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(54) RECEPTOR PROTEIN SPECIFICALLY RECOGNIZING BACTERIAL DNA

(57)Abstract:
PROBLEM TO BE SOLVED: To provide the receptor protein that specifically recognizes bacterial DNA bearing the non-methylated CpG sequence, the gene DNA coding the same and experimental model animals that are useful for examining the response of host immunocyte to bacterial infections.
SOLUTION: The DNA encoding the receptor protein that specifically recognizes the bacterial DNA bearing the non-methylated CpG sequence is screened by the BLAST search, a plurality of EST clones having high similarity to various kinds of TLS are screened and they are used as probes to isolate the full-length cDNA from the mouse macrophage cDNA library, the base sequence of the cDNA is analyzed to confirm that the TLR9 has the conserved areas such as the areas of LRR, TIR and the like. Then, the knockout mouse is established and the TLR9 is confirmed to be a receptor protein of the nucleotide including the non-methylated CpG sequence of the bacterial DNA.

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CLAIMS

[Claim(s)]
[Claim 1] DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array.
[Claim 2] DNA according to claim 1 to which receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array is characterized by being protein of the following (a) or (b).
(a) Protein which has reactivity to the bacterial DNA which 1 or some amino acid consist of deletion and an amino acid sequence permuted or added, and has a non-methylating CpG array in the amino acid sequence shown in the protein (b) array number 2 which consists of an amino acid sequence shown in the array number 2 [claim 3] DNA according to claim 1 characterized by including a part or all of these arrays in the base sequence shown in the array number 1, or its complementary sequence list.
[Claim 4] DNA according to claim 1 characterized by hybridizing under DNA which constitutes a gene according to claim 3, and stringent conditions.
[Claim 5] DNA according to claim 1 to which receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array is characterized by being protein of the following (a) or (b).
(a) Protein which has reactivity to the bacterial DNA which 1 or some amino acid consist of deletion and an amino acid sequence permuted or added, and has a non-methylating CpG array in the amino acid sequence shown in the protein (b) array number 4 which consists of an amino acid sequence shown in the array number 4 [claim 6] DNA according to claim 1 characterized by including a part or all of these arrays in the base sequence shown in the array number 3, or its complementary sequence list.
[Claim 7] DNA according to claim 1 characterized by hybridizing under DNA which constitutes a gene according to claim 6, and stringent conditions.
[Claim 8] Receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array.
[Claim 9] Protein according to claim 8 characterized by consisting of an amino acid sequence shown in the array number 2.
[Claim 10] Protein according to claim 8 characterized by 1 or some amino acid consisting of deletion and an amino acid sequence permuted or added in the amino acid sequence shown in the array number 2.
[Claim 11] Protein according to claim 8 characterized by consisting of an amino acid sequence shown in the array number 4.
[Claim 12] Protein according to claim 8 characterized by 1 or some amino acid consisting of deletion and an amino acid sequence permuted or added in the amino acid sequence shown in the array number 4.
[Claim 13] Claims 8-12 are fusion protein which combined protein, and the marker protein and/or the peptide tag of a publication either.
[Claim 14] Claims 8-12 are the antibodies specifically combined with the protein of a publication either.

[Claim 15] The antibody according to claim 14 characterized by an antibody being a monoclonal antibody.
[Claim 16] Claims 8-12 are the host cells which come to contain the manifestation system which can discover the protein of a publication either.
[Claim 17] The nonhuman animal characterized by the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array carrying out a superfluous manifestation.
[Claim 18] The nonhuman animal characterized by the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffering a loss on a chromosome.
[Claim 19] The nonhuman animal according to claim 18 characterized by being refractoriness to the bacterial DNA which has a non-methylating CpG array.
[Claim 20] Claims 17-19 to which the Rodentia animal is characterized by being a mouse are the nonhuman animals of a publication either.
[Claim 21] To the cell into which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, claims 1-7 are the preparation approaches of the cell which discovers the protein which has reactivity to the bacterial DNA which has the non-methylating CpG array characterized by introducing DNA of a publication either.
[Claim 22] The cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by being obtained by the preparation approach of the cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array according to claim 21.
[Claim 23] The screening approach of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by cultivating the cell which has discovered the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array by in vitro one under existence of a specimen material, and measuring and evaluating TLR9 activity, or an antagonist.
[Claim 24] The screening approach of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by to measure and evaluate the macrophage by which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array medicates with a specimen material the nonhuman animal which suffered a loss on the chromosome, and is obtained from this nonhuman animal, or the TLR9 activity of a spleen cell, or an antagonist.
[Claim 25] The screening approach of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by measuring and evaluating the macrophage by which the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array medicates with a specimen material the nonhuman animal which carried out the superfluous manifestation, and is obtained from this nonhuman animal, or the TLR9 activity of a spleen cell, or an antagonist.
[Claim 26] The screening approach of the agonist of the protein which has reactivity to the bacterial DNA which has the non-methylating CpG array according to claim 24 or 25 whose nonhuman animal is characterized by being a mouse, or an antagonist.
[Claim 27] either of claims 23-26 -- the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array acquired by the screening approach of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of a publication, or an antagonist, or an antagonist.
[Claim 28] The physic constituent which contains all of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, or its part as an active principle.
[Claim 29] The physic constituent which contains agonist or an antagonist according to claim 27 as an active principle.
[Claim 30] The diagnostic kit of the illness relevant to the deletion of the DNA array which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the

non-methylating CpG array characterized by including DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array in a specimen, and DNA according to claim 3 which can compare a base sequence with DNA according to claim 3, a permutation, and/or addition.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the genes of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, and this receptor protein, and those use.
[0002]
[Description of the Prior Art] It is known that a toll (Toll) gene is required for the decision (Cell 52, 269-279, 1988, Annu.Rev.Cell Dev.Biol.12, 393-416, 1996) of the dorso-ventral axis in the embryogenesis of drosophila and the antifungal immune response in an adult (Cell 86,973-983, 1996). This Toll is an I-beam film penetration acceptor which has a leucine rich repeat (LRR) to an extracellular field. This intracytoplasmic field It is clear that the intracytoplasmic field and homology of a mammals interleukin 1 acceptor (IL-1R) are high (). [Nature 351, 355-356, 1991,] [Annu.Rev.Cell Dev.Biol.] 12, 393-416, 1996, J.Leukoc.Biol.63, 650-657, 1998.
[0003] The homologue of the mammals of Toll called a Toll Mr. acceptor (TLR) is identified in recent years. By current [, such as TLR2 and TLR4,] Six families are reported (). [Nature] 388, 394-397, 1997, Proc.Natl.Acad.Sci.USA 95, 588-593, 1998, Blood 91, 4020-4027, 1998, Gene 231, 59-65, 1999. This TLR family minds MyD88 which is adapter protein like the above-mentioned IL-1R. Carry out the recruit of the IL-1R joint kinase (IRAK), and TRAF6 is activated. Activating down-stream NF-kappa B is known (J.Exp.Med.187, 2097-2101, 1998, Mol.Cell 2, 253-258, 1998, Immunity 11, 115-122, 1999). Moreover, it is thought that the role of the TLR family in the mammals is concerned with the immunological recognition native as a pattern recognition acceptor (PRR:pattern recognition receptor) which recognizes bacterial common structure (Cell 91, 295-298, 1997).
[0004] One of the pathogen associated-molecule patterns (PAMP:pathogen-associated molecular pattern) recognized by Above PRR It is lipopolysaccharide (LPS) which is the principal component of the adventitia of a gram negative (Cell 91, 295-298, 1997). This LPS stimulates a host cell. To a host cell TNFalpha, The various inflammatory cytokine of IL-1 and IL-6 grade is made to produce (457 28 Adv. Immunol. 293 -450, 1979, and Annu. Rev. Immunol. 13, 437- 1995), With LPS binding protein (LBP:LPS-bindingprotein) It is known that captured LPS will be handed over by CD14 on cell surface (Science 249, 1431-1433, 1990, Annu.Rev.Immunol.13, 437-457, 1995). It is unresponsive nature at LPS whose TLR4 knock AUTOUSU this invention persons produce the knockout mouse of TLR4, and is the principal component of the adventitia of the above-mentioned gram negative (J.Immunol.162, 3749-3752, and 1999), TLR2 knockout mouse was produced and it has reported that the reactivity over the peptidoglycan whose macrophages of TLR2 knockout mouse are a gram positive cell wall and its constituent falls (Immunity, 11, 443-451, and 1999).
[0005] On the other hand, an oligonucleotide including bacterial DNA (bacteria origin DNA) or a non-methylating CpG array The immunocyte of a mouse and Homo sapiens is stimulated (Trends Microbiol.4, 73-76, 1996, Trends Microbiol.6, 496-500, 1998), stimulating the T helper 1 cell (Th1) Mr. inflammatory response governed by emission of IL-12 and IFNgamma (EMBO J. -- 18, 6973-6982, 1999, J.Immunol.161, and 3042-3049 --) An oligonucleotide including a CpG array from 1998, Proc.Natl.Acad.Sci.USA 96, 9305-9310, and 1999 Cancer, As an adjuvant of the vaccine strategy containing the vaccine of allergy and an infectious disease ********** is advocated

(Adv.Immunol.73, 329-368, 1999, Curr.Opin.Immunol.12, 35-43, 2000, Immunity 11, 123-129, 1999). Thus, in spite of expecting effectiveness in clinical practical use, the molecule mechanism in which bacterial DNA including a non-methylating CpG array activates immunocyte is not known well.

[0006]

[Problem(s) to be Solved by the Invention] As mentioned above, although the bacteria origin DNA containing the CpG motif which is not methylated activates immunocyte very much and the response of Th1 is guided, the activity with the molecular level is seldom understood. The technical problem of this invention is to offer a useful experiment model animal, when investigating the responsibility of the member receptor protein TLR9 of the TLR family which recognizes specifically the bacterial DNA which has a non-methylating CpG array which can clarify the operation with the molecular level of an oligonucleotide including the non-methylating CpG array of bacterial DNA, DNA which carries out the code of it, and the host immunocyte to a bacterial infectious disease.

[0007]

[Means for Solving the Problem] The TLR family in the mammals concerned with the immunological recognition native as a pattern recognition acceptor which recognizes bacterial common structure By current Six members (TLR 1-6) are released (Nature 388, 394-397, 1997, Proc.Natl.Acad.Sci.USA, 95, 588-593, 1998, Gene 231, 59-65, 1999). Two new members, TLR7 and TLR8, are registered into GenBank (registration numbers AF240467 and AF246971). moreover, the perfect length cDNA is found out also about TLR9, and it registers with GenBank -- **** (registration number AF 245704) -- it was not known about the function.

[0008] this invention persons screened DNA which carries out the code of the member receptor protein of the TLR family which recognizes specifically the bacterial DNA which has a non-methylating CpG array by BLAST search, screened many sequence tag (EST) clones which have the already identified various kinds TLR and high similarity, used such gene fragmentation as the probe, and isolated cDNA which has perfect die length from a mouse macrophage cDNA library, and Homo sapiens cDNA isolated them using this. Next, the base sequence of these cDNA(s) was analyzed and it checked that it was TLR9 to which saved areas, such as LRR and a TIR field, exist in this TLR family. Then, this TLR9 knockout mouse is produced, and it shows clearly that it is the receptor protein of an oligonucleotide with which TLR9 includes the non-methylating CpG array of bacterial DNA, and came to complete this invention.

[0009] Namely, DNA (claim 1) to which this invention carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, The receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array In the amino acid sequence shown in the protein (b) array number 2 which consists of an amino acid sequence shown in the DNA(a) array number 2 according to claim 1 characterized by being protein of the following (a) or (b) 1 or some amino acid consist of deletion and an amino acid sequence permuted or added. And the protein (claim 2) which has reactivity to the bacterial DNA which has a non-methylating CpG array, DNA (claim 3) according to claim 1 characterized by including a part or all of these arrays in the base sequence shown in the array number 1, or its complementary sequence list, DNA (claim 4) according to claim 1 characterized by hybridizing under DNA which constitutes a gene according to claim 3, and stringent conditions, The receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array In the amino acid sequence shown in the protein (b) array number 4 which consists of an amino acid sequence shown in the DNA(a) array number 4 according to claim 1 characterized by being protein of the following (a) or (b) 1 or some amino acid consist of deletion and an amino acid sequence permuted or added. And the protein (claim 5) which has reactivity to the bacterial DNA which has a non-methylating CpG array, DNA (claim 6) according to claim 1 characterized by including a part or all of these arrays in the base sequence shown in the array number 3, or its complementary sequence list, It is related with DNA (claim 7) according to claim 1 characterized by hybridizing under DNA which constitutes a gene according to claim 6, and stringent conditions.

[0010] Moreover, the receptor protein (claim 8) which recognizes specifically the bacterial DNA in which this invention has a non-methylating CpG array, In the protein (claim 9) according to claim 8 characterized by consisting of an amino acid sequence shown in the array number 2, and the amino acid sequence shown in the array number 2 The protein (claim 10) according to claim 8 characterized

by 1 or some amino acid consisting of deletion and an amino acid sequence permuted or added, In the protein (claim 11) according to claim 8 characterized by consisting of an amino acid sequence shown in the array number 4, and the amino acid sequence shown in the array number 4 It is related with the protein (claim 12) according to claim 8 characterized by 1 or some amino acid consisting of deletion and an amino acid sequence permuted or added.

[0011] Claims 8-12 this invention either Moreover, the fusion protein (claim 13) which combined protein, and the marker protein and/or the peptide tag of a publication, Claims 8-12 either The antibody (claim 14) specifically combined with the protein of a publication, It is related with the antibody (claim 15) according to claim 14 characterized by an antibody being a monoclonal antibody, and the host cell (claim 16) which comes to contain the manifestation system which can discover any of claims 8-12, or the protein of a publication.

[0012] Moreover, the nonhuman animal (claim 17) characterized by the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array carrying out the superfluous manifestation of this invention, The nonhuman animal (claim 18) characterized by the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffering a loss on a chromosome, It is related with the nonhuman animal (claim 19) according to claim 18 characterized by being refractoriness to the bacterial DNA which has a non-methylating CpG array, and the nonhuman animal (claim 20) of claims 17-19 to which the Rodentia animal is characterized by what is been a mouse which is a publication either.

[0013] This invention into moreover, the cell into which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome Claims 1-7 either The preparation approach (claim 21) of the cell which discovers the protein which has reactivity to the bacterial DNA which has the non-methylating CpG array characterized by introducing DNA of a publication, By the preparation approach of the cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array according to claim 21 It is related with the cell (claim 22) which discovers the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by being obtained.

[0014] Moreover, this invention cultivates the cell which has discovered the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array by in vitro one under existence of a specimen material. The screening approach (claim 23) of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by measuring and evaluating TLR9 activity, or an antagonist, The nonhuman animal to which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome is medicated with a specimen material. The screening approach (claim 24) of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by measuring and evaluating the macrophage obtained from this nonhuman animal, or the TLR9 activity of a spleen cell, or an antagonist, The nonhuman animal in which the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array carried out the superfluous manifestation is medicated with a specimen material. The screening approach (claim 25) of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by measuring and evaluating the macrophage obtained from this nonhuman animal, or the TLR9 activity of a spleen cell, or an antagonist, A nonhuman animal is related with the screening approach (claim 26) of the agonist of the protein which has reactivity to the bacterial DNA which has the non-methylating CpG array according to claim 24 or 25 characterized by being a mouse, or an antagonist.

[0015] Moreover, this invention Claims 23-26 either by the screening approach of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of a publication, or an antagonist The agonist or the antagonist (claim 27) of receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array acquired, The physic constituent (claim 28) which contains all of the receptor protein which recognizes

specifically the bacterial DNA which has a non-methylating CpG array, or its part as an active principle, DNA which carries out the code of the physic constituent (claim 29) which contains agonist or an antagonist according to claim 27 as an active principle, and the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array in a specimen, The deletion of the DNA array which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array characterized by including DNA according to claim 3 which can compare a base sequence with DNA according to claim 3, It is related with the diagnostic kit (claim 30) of the illness relevant to a permutation and/or addition.

[0016]

[Embodiment of the Invention] As bacterial DNA which has a non-methylating CpG array in this invention Immunocytes, such as a T cell, a B cell, and an antigen presenting cell, can be activated, and an immune response can be guided. As long as it is DNA originating in bacteria, such as oligodeoxynucleotide (ODN) which has the CpG motif which is not methylated, what kind of thing may be used. ESERISHIA KORI, the Klebsiella pneumoniae, Pseudomonas AERUGINOSA, Salmonella typhimurium, Serratia marcescens, the Shigella flexneri, Vibrion KOREREE, Salmonella Minnesota, Porphyromonas gingivalis, DNA of the bacteria origins, such as Staphylococcus aureus, the Corynebacterium diphtheria, Nocardia KOERIAKA, and streptococcus new MONIA, can be mentioned concretely.

[0017] As receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array It is not what will be restricted especially if it is protein which can recognize specifically the bacterial DNA which has a non-methylating CpG array. For example, it sets to the amino acid sequence shown by TLR9 and the array number 2 of the Homo sapiens origin shown by the array number 2 of an array table. The protein which can recognize specifically the bacterial DNA which 1 or some amino acid consist of deletion and an amino acid sequence permuted or added, and has the above-mentioned non-methylating CpG array, and these recombination protein can be mentioned concretely. The receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array can be prepared by the well-known approach based on the DNA array information etc.

[0018] moreover, as DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention In DNA which carries out the code of TLR9 of the Homo sapiens origin shown by the array number 2 of an array table, for example, the thing shown by the array number 1, and the amino acid sequence shown by the array number 2 1 or some amino acid consist of deletion and an amino acid sequence permuted or added. And DNA which carries out the code of the protein which can recognize specifically the bacterial DNA which has the above-mentioned non-methylating CpG array, DNA which carries out the code of the protein which can recognize specifically the bacterial DNA which hybridizes under these DNA and stringent conditions, and has the above-mentioned non-methylating CpG array is also included. These can be prepared by the well-known approach in TLR9 of the mouse origin based on the DNA array information etc. from a mouse RAW264.7cDNA library, 129 / SvJ mouse gene library, etc.

[0019] moreover, the thing for which the DNA which performs hybridization under stringent conditions to the DNA library of the mouse origin at the base sequence shown in the array number 1 or its complementary sequence list by using a part or all of these arrays as a probe, and hybridizes to this probe isolates -- receptor protein TLR9 -- said -- the DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the immunity induction non-methylating CpG array made into the **** purpose can also obtain. As conditions for the hybridization for acquiring this DNA, washing processing at 42 degrees C by 42-degree C hybridization and 1xSSC, and the buffer solution containing 0.1% of SDS can be mentioned, for example, and washing processing at 65 degrees C by 65-degree C hybridization and 0.1xSSC, and the buffer solution containing 0.1% of SDS can be mentioned more preferably. In addition, as an element which affects the stringency of hybridization, there are various elements in addition to the above-mentioned temperature conditions, and if it is this contractor, it is possible to realize stringency equivalent to the stringency of the hybridization which carried out [above-mentioned] instantiation, combining various elements suitably.

[0020] To the receptor protein which recognizes specifically the bacterial DNA which has non-methylating CpG arrays, such as a mouse and Homo sapiens, to be fusion protein of this invention What combined marker protein and/or a peptide tag is said. As marker protein As long as it is marker protein known conventionally, what kind of thing may be used. for example, can mention concretely alkaline phosphatase, Fc field of an antibody, HRP, GFP, etc., and as a peptide tag in this invention The peptide tag known conventionally [, such as a Myc tag, a His tag, a FLAG tag and a GST tag,] can be illustrated concretely. This fusion protein is useful also as purification of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array which could produce with the conventional method and used the compatibility of nickel-NTA and a His tag, detection of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, the quantum of an antibody to the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, and a reagent for research of this other these field.

[0021] As an antibody specifically combined with the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention A monoclonal antibody, a polyclonal antibody, a chimeric antibody, a single strand antibody, immunity, such as a hominization antibody, -- a specific antibody being mentioned concretely, and, although these can produce the bacterial DNA which has the above-mentioned non-methylating CpG array with a conventional method, using as an antigen the receptor protein recognized specifically A monoclonal antibody is more desirable in respect of the singularity also in it. a diagnosis of the illness to which the antibody specifically combined with the receptor protein which recognizes specifically the bacterial DNA which has non-methylating CpG arrays, such as this monoclonal antibody, originates in the variation or deletion of TLR9, and a controlled part of TLR9 -- a cordless handset -- it is useful when clarifying a style.

[0022] The antibody to the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array The fragment containing the receptor protein or the epitope which recognizes specifically the bacterial DNA which has this non-methylating CpG array to an animal (preferably except Homo sapiens) using the protocol of common use, It is produced by prescribing for the patient the cell which discovered this protein on the film front face. Or for preparation of a monoclonal antibody The hybridoma method for bringing about the antibody produced with the culture of continuation cell lineage (Nature 256, 495-497, 1975), The TORIOMA method, a Homo sapiens B cell hybridoma method (ImmunologyToday 4, 72, 1983) and the EBV-hybridoma method (MONOCLONAL ANTIBODIES AND CANCER THERAPY and pp.77-96 --) The approach of arbitration, such as Alan R.Liss, Inc., and 1985, can be used. As receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array below, the production approach of of the monoclonal antibody which mentions TLR9 of the mouse origin as an example, and is specifically combined to TLR9 of the mouse origin, i.e., anti-mTLR9 monoclonal antibody, is explained.

[0023] The above-mentioned anti-mTLR9 monoclonal antibody is producible by cultivating an anti-mTLR9 monoclonal-antibody production hybridoma with a conventional method by in vivo one or in vitro one. For example, in an in vivo system, it can obtain by cultivating by the culture medium for animal cell culture in an in vitro system a rodent and by cultivating by intraperitoneal [of a mouse or a rat] preferably again. As a culture medium for cultivating a hybridoma by the in vitro system, cell culture media, such as RPMI1640 containing antibiotics, such as streptomycin and penicillin, or MEM, can be illustrated.

[0024] An anti-mTLR9 monoclonal-antibody production hybridoma **** a BALB/c mouse using the receptor protein TLR9 obtained from the mouse etc., and can create an anti-mTLR9 monoclonal-antibody production hybridoma by carrying out the cell fusion of the spleen cell of a mouse and mouse NS-1 cell (ATCC TIB-18) by which immunity was carried out with a conventional method, and screening them with an immunofluorescenct-stain pattern. Moreover, if it is the approach generally used to purification of protein as the separation / purification approach of this monoclonal antibody, what kind of approach may be used and liquid chromatography, such as affinity chromatography, can be illustrated concretely.

[0025] Moreover, in order to build the single strand antibody to the receptor protein which recognizes

specifically the bacterial DNA which has the above-mentioned non-methylating CpG array of this invention, the method of preparation (U.S. Pat. No. 4,946,778) of a single strand antibody is applicable. Moreover, in order to make a hominization antibody discover, a transgenic mouse or other mammalians can be used, the clone which discovers the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array can be isolated and identified using the above-mentioned antibody, or affinity chromatography can also refine the polypeptide. The antibody to the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array is useful when clarifying the molecule device of receptor protein in which the bacterial DNA which has a non-methylating CpG array is recognized specifically.

[0026] To antibodies, such as the above-mentioned anti-mTLR9 monoclonal antibody, moreover, for example, fluorescent materials, such as FITC (fluorescein isocyanate) or tetramethyl rhodamine isocyanate, The radioisotope of 125I, 32P, 35S, or 3H grade, and the alkaline phosphatase, By using the fusion protein with which what carried out the indicator with enzymes, such as a peroxidase, beta-galactosidase, or a phycoerythrin, firefly luminescence protein, such as Green fluorescence protein (GFP), etc. were united Functional analysis of the receptor protein which recognizes specifically the bacterial DNA which has the above-mentioned non-methylating CpG array can be performed. Moreover, as an immunological measuring method, approaches, such as the RIA method, the ELISA method, a fluorescent antibody technique, a plaque technique, the spot method, an erythrocyte agglutination method, and an Ouchterlony method, can be mentioned.

[0027] This invention relates to the host cell which comes to contain the manifestation system which can discover the receptor protein which recognizes specifically the bacterial DNA which has the above-mentioned non-methylating CpG array again. The installation to the host cell of the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array Davis et al. () [BASIC METHODS] IN MOLECULAR BIOLOGY, 1986, and Sambrook et al. () [MOLECULAR] CLONING: A LABORATORY the approach indicated by many standard laboratory manuals, such as MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold SpringHarbor, N.Y., and 1989, -- for example Calcium phosphate transfection, DEAE-dextran medium transfection, Transformer BEKUSHON (transvection), a microinjection, Cationic lipid medium transfection, electroporation, transduction, scrape loading (scrape loading) Projectile installation (ballistic introduction), infection, etc. can perform. And as a host cell, animals-and-plants cells, such as fungus cells, such as bacteria prokaryotic cells, such as Escherichia coli, Streptomyces, a Bacillus subtilis, a streptococcus, and Staphylococcus, and yeast, an Aspergillus, the insect cell of Drosophila S2 and Spodoptera Sf9 grade, an L cell and a CHO cell, a COS cell, a HeLa cell, C127 cell, a BALB/c 3T3 cell (the variant which suffered a loss in dihydrofolate reductase, a thymidine kinase, etc. is included), a BHK-21 cell, HEK293 cell, a Bowes melanoma cell, and oocyte etc. can be mentioned.

[0028] Moreover, as long as it is the manifestation system which can make the receptor protein which recognizes specifically the bacterial DNA which has the above-mentioned non-methylating CpG array as a manifestation system discover by host intracellular, what kind of thing may be used. The manifestation system originating in a chromosome, episome, and a virus, for example, the bacterial plasmid origin, The yeast plasmid origin, papovavirus like SV40, a vaccinia virus, Adenovirus, the fowlpox virus, the pseudorabies virus, the vector of the retrovirus origin, The vector originating in the bacteriophage origin, the transposon origins, and these combination, for example, the thing originating in the hereditary element of cosmid, a plasmid like phagemid, and a bacteriophage, can be mentioned. Not only making a manifestation cause but these manifestation system may include the control array which adjusts a manifestation.

[0029] The receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array which cultivates the cell membrane of the host cell which comes to contain the above-mentioned manifestation system, or this cell, and this cell, and is acquired can be used for the screening approach of this invention so that it may mention later. For example, as an approach of obtaining a cell membrane, F.Pietri-Rouxel's and others (Eur.J.Biochem., 247, 1174-1179, 1997) approach etc. can be used. Moreover, in order to collect and refine the receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array from a cell culture object An ammonium sulfate or ethanol precipitate, an acid extract, an anion, or a cation

exchange chromatography, A phosphocellulose chromatography, hydrophobic interaction chromatography, a well-known approach including affinity chromatography, hydroxyapatite chromatography, and a lectin chromatography -- high performance chromatography is used preferably. As a column especially used for affinity chromatography For example, the column which combined the receptor protein antibody which recognizes specifically the bacterial DNA which has anti-** methylation CpG arrays, such as anti-TLR9 monoclonal antibody, When the usual peptide tag is added to the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of the above-mentioned TLR9 grade By using the column which combined the matter which has compatibility in this peptide tag, the receptor protein which recognizes specifically the bacterial DNA which has these non-methylating CpG arrays can be obtained.

[0030] With the nonhuman animal in which the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the above-mentioned non-methylating CpG array in this invention carries out a superfluous manifestation The nonhuman animal which produces the receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array compared with a wild type nonhuman animal in large quantities is said. With moreover, the nonhuman animal by which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome A part or all of a gene that carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array on a chromosome is inactivated by gene variation, such as destruction, a deficit, and a permutation. A ******** nonhuman animal is said for the function which discovers the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array. And as a nonhuman animal in this invention, although nonhuman animals, such as the Rodentia animals, such as a rabbit, and a mouse, a rat, can be mentioned concretely, it is not limited to these.

[0031] Moreover, refractoriness means the reactivity of the cell which constitutes the living body or living body to the stimulus by bacterial DNA, an organization, or an organ falling, or being lost mostly to the bacterial DNA which has a non-methylating CpG array in this invention. Therefore, the nonhuman animal of refractoriness means animals other than Homo sapiens, such as a mouse which the reactivity of the cell which constitutes a living body or a living body, an organization, or an organ is falling, or is lost mostly, a rat, and a rabbit, to the stimulus by bacterial DNA to the bacterial DNA which has a non-methylating CpG array in this invention. Moreover, as a stimulus by bacterial DNA, the in vivo stimulus which medicates a living body with bacterial DNA, the in vitro stimulus which contacts bacterial DNA into the cell separated from the living body can be mentioned, and, specifically, the nonhuman animal to which TLR9 gene functions, such as TLR9 knockout mouse, suffered a loss on the chromosome can be mentioned.

[0032] by the way, to the homozygote nonhuman animal born according to Mendel's laws The receptor protein deficit mold or superfluous manifestation mold which recognizes specifically the bacterial DNA which has a non-methylating CpG array, and the wild type of the brood are contained. From the ability of exact comparative experiments to be carried out on individual level by using the deficit mold or superfluous manifestation mold in a these homozygote nonhuman animal, and the wild type of the brood for coincidence The nonhuman animal of a wild type, i.e., the nonhuman animal to which the gene function which carries out a code suffers a loss or discovers [superfluous] the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array on a chromosome and an animal of the same kind, It is desirable to use the animal of a brood together on the occasion of screening of this invention indicated below furthermore. The gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array explains below the bacterial DNA which has a non-methylating CpG array for the production approach of the nonhuman animal which carries out a deficit or a superfluous manifestation taking the case of the knockout mouse and transgenic mouse of receptor protein which are recognized specifically on a chromosome.

[0033] For example, the mouse with which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of TLR9 grade suffered a loss on the chromosome, Namely, the receptor protein knockout mouse which recognizes specifically the bacterial DNA which has a non-methylating CpG array The

gene fragment obtained from the mouse gene library by approaches, such as PCR, is used. The gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the above-mentioned non-methylating CpG array is screened. The subclone of the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the screened non-methylating CpG array is carried out using a virus vector etc., and it specifies by DNA sequencing. A target vector is produced by permuting all or a part of gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this clone by a pMC1 neo gene cassette etc., and introducing genes, such as a diphtheria toxin A fragmentation (DT-A) gene and a thymidine kinase (HSV-tk) gene of a herpes simplex virus, into 3' end side.

[0034] this produced targeting vector -- a line ---izing -- electroporation (electric punching) -- it introduces into an embryonic stem cell by law etc., homonous recombination is performed, and the embryonic stem cell which caused homonous recombination with antibiotics, such as G418 and gun cyclo beer (GANC), is chosen from those homonous recombinant. Moreover, it is desirable to check whether it is the recombinant which this selected embryonic stem cell makes the purpose with a Southern blot technique etc. The microinjection of the clone of the checked embryonic stem cell is carried out into the blastocyst of a mouse, this blastocyst is returned to the mouse of assumed parents, and a chimeric mouse is produced. If this chimeric mouse is made INTAKUROSU [mouse / of a wild type], the receptor protein knockout mouse which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention is producible by being able to obtain a heterozygote mouse and making this heterozygote mouse INTAKUROSU. Moreover, as an approach of checking whether the receptor protein knockout mouse which recognizes specifically the bacterial DNA which has a non-methylating CpG array having occurred, it investigates with a Northern blot technique etc., and there is a method of isolating RNA from the mouse obtained by the above-mentioned approach, and investigating the manifestation of this mouse by western blotting etc., for example.

[0035] That moreover, it is unresponsive nature to the bacterial DNA in which TLR9 created knockout mouse has a non-methylating CpG array For example, CpG ODN The macrophage of TLR9 knockout mouse, Immunocytes, such as a mononuclear cell and a dendritic cell, are made to contact by in vitro one or in vivo one. The amounts of production, such as TNF-alpha in this cell, IL-6, IL-12, and IFN-gamma, It can check by measuring activation of the molecule in the signal transfer path of TLR(s)9, such as the amount of manifestations of antigens, such as a growth response of a spleen B cell, and CD40, CD80 and CD86 in a spleen B cell front face, the MHC class II, and NF-kappa B and JNK, IRAK. And TLR9 knockout mouse of this invention can be used as a model useful to the vaccine development to an elucidation and bacterial infection of action mechanisms, such as bacterial DNA which has a non-methylating CpG array.

[0036] The transgenic mouse of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array The receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of TLR9 grade to cDNA which carries out a code A chicken beta-actin, Promotors, such as mouse neurofilament and SV40, and a rabbit beta globin, Unite poly A or the introns, such as SV40, and an introductory gene is built. The microinjection of this introductory gene is carried out to the pronucleus of a mouse fertilized egg. After cultivating the obtained ootid, it can transplant to the uterine tube of the mouse of assumed parents, and this transgenic mouse can be invented by breeding a transplanted animal after that and choosing the ** mouse which has said cDNA from the produced ** mouse. Moreover, selection of the ** mouse which has cDNA can be performed by the dot hybridization method which uses as a probe the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array which extracted and introduced rough DNA from the tail of a mouse etc., the PCR method using a specific primer, etc.

[0037] Moreover, if all or a part of DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention is used, a cell effective in gene therapies, such as deletion of receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, or an illness which originates unusually, can be prepared. As the preparation approach of these cells in this invention Into the cell

into which the gene function which carries out a code suffered a loss on the chromosome, the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array All or a part of DNA of above-mentioned this invention is introduced with transfection etc. The approach of obtaining the cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array can be mentioned. It is desirable to use the cell which Above DNA etc. is integrated by the chromosome and shows TLR9 activity to a stay bull as a cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has this non-methylating CpG array especially.

[0038] And DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the above-mentioned non-methylating CpG array again, The antibody to the receptor protein which recognizes specifically the bacterial DNA which has the fusion non-methylating CpG array which combined the receptor protein, the marker protein, and/or the peptide tag which recognize specifically the bacterial DNA which has a non-methylating CpG array, The host cell which comes to contain the manifestation system which can discover the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, The nonhuman animal in which the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array carries out a superfluous manifestation, The nonhuman animal to which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, If the cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array is used The reactant inhibitor or reactant promoting agent to the agonist or the antagonist of receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention, and the bacterial DNA which has a non-methylating CpG array can be screened. In the inhibitor or promoting agent to the microbism, the inhibitor and preventive to an allergic disease or cancer, or a remedy, gene therapy, etc., it may be the matter useful for a diagnosis and therapies, such as an inhibitor or an inhibitor, and deletion of TLR9 activity or an illness which originates unusually, which was obtained by these screening about a side effect.

[0039] The TLR9 above-mentioned activity reacts specifically with the bacterial DNA which has a non-methylating CpG array, and means the function to transmit a signal to intracellular. As a signal communicative function The function which produces cytokine, such as TNF-alpha, IL-6, IL-12, and IFN-gamma, The function which produces nitrite ion, the function to increase a cell, and the function which discovers antigens, such as CD40, CD80, CD86, and the MHC class II, in cell surface, Although the function to activate the molecule in the signal transfer path of TLR(s)9, such as NF-kappa B and JNK and IRAK, etc. can be illustrated concretely, it is not limited to these.

[0040] As the screening approach of the agonist of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention, or an antagonist Immunocytes, such as the bottom of existence of a specimen material, a macrophage, a spleen cell, or a dendritic cell, The cell which has discovered the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, The cell which has discovered the protein which has reactivity to the bacterial DNA which has non-methylating CpG arrays, such as a cell which discovers the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, is cultivated by in vitro one. The approach of measuring and evaluating TLR9 activity, and a wild type nonhuman animal, the nonhuman animal, to which the gene function which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, Or the nonhuman animal in which the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array carried out the superfluous manifestation is medicated with a specimen material. The approach of measuring and evaluating the TLR9 activity of immunocytes, such as a macrophage obtained from this nonhuman animal, a spleen cell, or a dendritic cell, etc. can be mentioned concretely.

[0041] Moreover, since facing measuring and evaluating extent of macrophage activity or spleen cell activity, and comparing and estimating it as the measured value of a wild type nonhuman animal, especially the wild type nonhuman animal of a brood as contrast can abolish the variation by

individual difference, it is desirable. This is the same also in screening of the reactant inhibitor or promoting agent to the bacterial DNA which has the non-methylating CpG array shown below.
[0042] moreover, as the screening approach of of the reactant inhibitor or promoting agent to the bacterial DNA which has a non-methylating CpG array The protein which has reactivity to the bacterial DNA which has a non-methylating CpG array under existence with a specimen material and the bacterial DNA which has a non-methylating CpG array, Or the incubation of the cell membrane which has discovered this protein is carried out by in vitro one. The approach of measuring and evaluating reactivity with this protein, After making the macrophage or spleen cell obtained from the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, and a specimen material contact by in vitro one beforehand, This macrophage or a spleen cell is cultivated under existence of the bacterial DNA which has a non-methylating CpG array. The approach of measuring and evaluating extent of this macrophage, the macrophage activity of a spleen cell, or spleen cell activity, After making the macrophage or spleen cell obtained from the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, and the bacterial DNA which has a non-methylating CpG array contact by in vitro one beforehand, The approach of cultivating this macrophage or a spleen cell under existence of a specimen material, and measuring and evaluating extent of this macrophage, the macrophage activity of a spleen cell, or spleen cell activity, After medicating beforehand with a specimen material the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, The macrophage or spleen cell obtained from this nonhuman animal is cultivated under existence of the bacterial DNA which has a non-methylating CpG array. The approach of measuring and evaluating extent of this macrophage, the macrophage activity of a spleen cell, or spleen cell activity, After medicating beforehand with a specimen material the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, The approach of measuring and evaluating extent of the macrophage which this nonhuman animal is infected with bacteria and obtained from this nonhuman animal, the macrophage activity of a spleen cell, or spleen cell activity, After infecting beforehand the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome with bacteria, The macrophage or spleen cell obtained from this nonhuman animal is cultivated under existence of a specimen material. The approach of measuring and evaluating extent of this macrophage, the macrophage activity of a spleen cell, or spleen cell activity, After infecting beforehand the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome with bacteria, The approach of measuring and evaluating extent of the macrophage which medicates this nonhuman animal with a specimen material, and is obtained from this nonhuman animal, the macrophage activity of a spleen cell, or spleen cell activity, After medicating beforehand with a specimen material the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome, The approach of infecting this nonhuman animal with bacteria, and measuring and evaluating extent of the macrophage activity in this nonhuman animal, or spleen cell activity, After infecting beforehand the nonhuman animal to which the gene function which carries out the code of the protein which has reactivity to the bacterial DNA which has a non-methylating CpG array suffered a loss on the chromosome with bacteria, This nonhuman animal can be medicated with a specimen material, and the approach of measuring and evaluating extent of the macrophage activity in this nonhuman animal or spleen cell activity etc. can be mentioned concretely. Moreover, as bacterial DNA which has the non-methylating CpG array used for these screening approaches, it is CpG. Although it is desirable to use ODN (TCC-ATG-ACG-TTC-CTG-ATG-CT: array number 5), it is not limited to this, either.
[0043] The bacterial DNA which has the non-methylating CpG array which consists of comparing with the DNA array which carries out the code of the receptor protein which recognizes specifically the

bacterial DNA which has the non-methylating CpG array of this invention for the DNA array which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array in a specimen again is related in this invention to the diagnostic kit used for the diagnosis of the illness relevant to the activity of receptor protein or the manifestation recognized specifically. It is effective to the diagnosis of the illness produced by the too little manifestation of receptor protein which recognizes specifically the bacterial DNA which can perform detection of the variant of DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array by finding out the individual which has variation in a gene on DNA level, and has a non-methylating CpG array, a superfluous manifestation, or variation manifestation. Although the genomic DNA which can be obtained from biopsies, such as a test subject's cell, for example, blood, urine, saliva, and an organization, and RNA or cDNA can be concretely mentioned as a specimen used for this detection, when it is not limited to these and uses this specimen, what was amplified by PCR etc. can also be used. And change of the size of the magnification product when comparing with normal genotype can detect the deletion and insertion mutation of a base sequence, and point mutation can identify the receptor protein which recognizes specifically the bacterial DNA which has an indicator non-methylating CpG array for Magnification DNA by making it hybridize with the gene which carries out a code. Thus, the diagnosis or judgment of the illness relevant to the activity of receptor protein or the manifestation which recognizes specifically the bacterial DNA which has a non-methylating CpG array can be carried out by detecting the variation of the gene which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array.

[0044] This invention again A non-methylating CpG array The activity of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array which consists of all or a part of DNA which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which it has, or RNA of antisense strands, or the probe for a diagnosis of the disease relevant to a manifestation, And the antibody specifically combined with the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of the probe concerned and/or this invention is contained. It is related with the diagnostic kit of the disease relevant to the activity of receptor protein or the manifestation which recognizes specifically the bacterial DNA which has the becoming non-methylating CpG array. It is all or a part of DNA (cDNA) which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array as said probe for a diagnosis, or RNA (cRNA) of antisense strands, and it will not be restricted especially if it has the die length (at least 20 or more bases) of extent materialized as a probe. In order to make the antibody specifically combined with the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this probe and/or this invention into the active principle of the diagnostic drug of the disease of symptoms, such as microbism, it is desirable to dissolve in suitable buffers by which a probe is not disassembled, and sterilized water. Moreover, the immunity staining technique (Dev.Biol.170, 207-222, 1995, J. Neurobiol. 29, 1-17, 1996) using these diagnostic drugs, an In situ hybridization method (J. Neurobiol. 29, 1-17, 1996), and in situ The disease of symptoms, such as microbism, can also be diagnosed by approaches, such as the PCR method.

[0045] As long as all of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of TLR9 grade as a physic constituent of this invention or its part, the agonist of the above-mentioned receptor protein, and an antagonist are included, what kind of thing may be used. Specifically, the conquest agent, inhibitor, inhibitor, etc. of the side effect by existence of the CpG motif acting as a failure in the therapy and gene therapy using the vaccine to the microbism, the vaccine to cancer, the remedy of allergosis including bronchial asthma, and an antisense oligonucleotide can be mentioned.

[0046] As mentioned above, the deletion of the DNA array which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has the non-methylating CpG array of this invention, As a diagnostic kit of the illness relevant to a permutation and/or addition As long as DNA which carries out the code of TLR9 is included, what kind of thing may be used. By comparing a base sequence with DNA which carries out the code of the receptor protein which recognizes specifically DNA which carries out the code of this TLR9, and the bacterial DNA which has a

non-methylating CpG array in a specimen A diagnosis of the deletion of the DNA array which carries out the code of the receptor protein which recognizes specifically the bacterial DNA which has a non-methylating CpG array, a permutation and/or the illness relevant to addition, for example, cancer, allergy, an infectious disease, etc. is attained.

[0047]

[Example] Although an example is given to below and this invention is explained to it still more concretely, the technical range of this invention is not limited by these examples.

Example 1 (cloning of TLR9)

As a result of searching GenBank using Homo sapiens's TLR4 DNA array information, homology found out the very high mouse EST (registration number AA 273731; mouse). The mouse RAW264.7cDNA library was screened by having used the PCR magnification product of this mouse EST as the probe, and the cDNA clone of perfect length shown in the array number 3 containing TLR9 perfect open reading frame was isolated. GenBank was searched using the DNA array information on this mouse TLR9, and the human genome array which has high homology was found out. Based on this human genome array, the cDNA edge was amplified and cDNA of Homo sapiens TLR9 of the perfect length who has the base sequence shown in the array number 1 was isolated from U937 cell (J.Immunol.163, 5039-5048, 1999). [0048] Example 2 (production of TLR9 knockout mouse)

TLR9 genomic DNA was isolated from the 129-/SvJ mouse gene library (Stratagene make), the subclone was carried out in the pBluescript II SK(+) vector (Stratagene make), and it specified by restriction enzyme mapping and DNA sequencing. The targetting vector permuted the fragmentation of 1.0kbs which carry out the code of a part of LRR (leucine rich repeat) field by the neomycin resistance gene cassette (pMC1-neo; Stratagene make), and built it by inserting a herpes simplex virus thymidine kinase (HSV-TK) as a negative selective marker (drawing 1). This targetting vector was line-ized, erection PORESHON was carried out, 292 clones which show resistance to G418 and gun cyclo beer were chosen as the day [of viviparity / 14.1st] embryonic stem cell (embryonic stem cell), and 14 clones were screened with the PCR method and a Southern blot technique.

[0049] The microinjection of the three target ES clones containing mutation TLR9 allele was carried out into the blastocyst of C57BL/6 mouse, and the chimeric mouse was produced. The chimeric mouse of this male was made to cross with C57BL / 6 female mouse, heterozygote F1 mouse was produced, and the homozygote mouse (TLR9 knockout mouse: TLR9-/-) was obtained by INTAKUROSU [this heterozygote F1 mouse] (drawing 2). In addition, the check of a homozygote mouse digested each genomic DNA extracted from the tail of a mouse by ScaI, and was performed with the Southern blot technique using the probe shown in drawing 1 . TLR9 knockout mouse (TLR9-/-) of this invention could be produced according to Mendel's laws, and did not show remarkable abnormalities till the 12th week.

[0050] In order to check that inactivation of TLR9 gene has occurred by mutation, Apply to electrophoresis all RNA (10microg) extracted from the spleen cell of a wild type mouse (+/+) and TLR9 knockout mouse (-/-), and it moves to the nylon film. Northern blot analysis was performed to C-end fragmentation, N-end fragmentation, or beta-actin (beta-actin) of TLR9 which carried out the indicator using specific cDNA by [32P] (drawing 3 R> 3). N-end fragmentation of these results to TLR9mRNA was not detected from the spleen cell of TLR9 knockout mouse. Moreover, when C-end fragmentation was used as a probe, although the thing of the wild type mouse origin and the thing of the almost same size were detected, in the volume, it turned out that the imprint of the mutant-mouse origin of Tlr9 is few. Then, RT-PCR method was performed using mRNA of the spleen cell obtained from the mutant mouse, and sequence analysis of the obtained product was performed. Consequently, it turned out that the neo gene is contained in Tlr9 imprinted gene, a stop codon appears at least in N-end of TLR9, and functional TLR9 protein is not discovered in a mutant mouse with this insertion of neo (drawing 4). In addition, the extraordinary component was not seen as a result of measuring the lymph cell of TLR9 knockout mouse by flow cytometry.

[0051] Example 3 (preparation of a peritoneal macrophage)

It pours 2ml (product made from DIFCO) of 4% of thioglycollate media into intraperitoneal [of a wild type mouse (wild-type) and TLR9 knockout mouse (TLR9-/-) / each] at a time. Isolate a peritoneum exudate cell from intraperitoneal [of each mouse] three days after, and these cells are cultivated at 37 degrees C for 2 hours in the RPMI1640 culture medium (product made from GIBCO) which added

10% of foetal calf serum (product made from GIBCO). By washing with the Hanks buffer solution (product made from Hank's buffered salt solution:HBSS;GIBCO) of an ice temperature, the non-adherent cell was removed and it was used for the following experiments by making an adherent cell into a peritoneal macrophage.

[0052] Example 4 (responsibility over the bacterial DNA which has the non-methylating CpG array of TLR9 knockout mouse)

Recently, CpG It became clear to depend for the responsibility of ODN (oligodeoxynucleotide) on MyD88 which is adapter protein in the signal transfer path through TLR. This MyD88 knockout mouse is CpG. Although not answered to ODN, TLR2 knockout mouse and TLR4 knockout mouse are CpG normally. It answers to ODN. These things are CpG. It is shown that ODN is recognized by TLR(s) other than TLR2 and TLR4. Then, CpG of TLR9 knockout mouse The responsibility over ODN was investigated. First, the amount of production of the inflammatory cytokine in a peritoneal macrophage was measured as follows.

[0053] CpG of the various concentration shown under existence of INFgamma (30 unit/ml) or nonexistence at drawing 5 in each peritoneal macrophage prepared according to the example 3 It cultivated for 24 hours together with ODN (0.1 or the product made from 1.0microM;TIB MOLBIOL; TCC-ATG-ACG-TTC-CTG-ATG-CT), PGN (the product made from 10microg/ml;Sigma and Fluka; Staphylococcus-aureus origin), and LPS (the product made from 1.0microg/ml;Sigma; Salmonella Minnesota Re-595 origin). TNFalpha after culture and in a culture supernatant, IL-6, and IL-12 Each concentration of p40 was measured by the ELISA method. This result is shown in drawing 5 . The macrophage of these results to a wild type mouse (Wild-type) is CpG. ODN is answered, TNFalpha, IL-6, and IL-12 are produced, and they are IFNgamma and CpG further. When stimulated by ODN, it turned out that the amount of production of TNFalpha, IL-6, and IL-12 increases. However, for the macrophage of the TLR9 knockout-mouse (TLR9-/-) origin, even the bottom of existence of IFNgamma is CpG. The inflammatory cytokine of detectable level was not produced in the response to ODN. Moreover, as for the macrophage of a wild type mouse and the TLR9 knockout-mouse origin, it turned out that comparable production of TNFalpha, IL-6, and IL-12 is mostly carried out by the response to LPS or PGN (drawing 5). In addition, each experimental result shows the average of n= 3. It is shown that N.D. in drawing was undetectable.

[0054] Moreover, CpG It investigated about the responsibility of the spleen cell of the wild type mouse (Wild-type) to ODN or LPS, and TLR9 knockout mouse (TLR9-/-). CpG of the various concentration which isolates the spleen cell (1x105) of each mouse, and is shown in drawing 6 It cultivated within 96 well plate by ODN or LPS, and the spleen cell was stimulated. 40 hours after culture, 1microcurie [3H]-thymidine (product made from DEYUPONTO) was added, it cultivated for further 8 hours, and the intake of [3H] was measured with beta scintillation counter (Packard make) (drawing 6). At the spleen cell of this result to a wild type mouse, it is CpG. Although the cell proliferation reaction was promoted depending on the dose of ODN or LPS, at the spleen cell of TLR9 knockout mouse, it is CpG of what kind of concentration. It also sets to an ODN stimulus and is CpG. The cell proliferation reaction by ODN was not seen. Moreover, CpG ODN was answered and the manifestation of the major histocompatibility complex (MHC) class II on the front face of a B cell of the wild type mouse origin increased. However, at the B cell of the TLR9 knockout-mouse origin, it is CpG. The increment in the manifestation of the MHC class II guided to ODN was not seen. The macrophage and B cell of the above thing to TLR9 knockout mouse are CpG. It turned out that it is specifically lacking in the responsibility over ODN.

[0055] Next, CpG The bacteria origin DNA containing ODN stimulates a dendritic cell potentially, and supporting development of Th1 cell is known (EMBO J.18, 6973-6982, 1999, J.Immunol.161, 3042-3049, 1998, Proc.Natl.Acad.Sci.USA 96, 9305-9310, 1999). Then, CpG The up-regulation of the surface molecule of production of ODN induction cytokine and the dendritic cell of the bone marrow origin was analyzed. The bone marrow cell of a wild type mouse (Wild-type) or TLR9 knockout mouse (TLR9-/-) It cultivates by RPMI1640 culture medium which added 10% of foetal calf serum containing a 10 ng(s)/ml mouse granulocyte macrophage colony-stimulating factor (product made from Peprotech) (J.Exp.Med.176, 1693-1702, 1992). Immature dendritic cells will be collected after culture on the 6th, and it is CpG of 0.1microM. It cultivated for two days in the RPMI1640 culture medium which added 10% of foetal calf serum under existence of ODN or 0.1microg [/ml] LPS or

nonexistence. IL-12 after culture and in supernatant liquid The concentration of p40 was measured by the ELISA method (drawing 7). The dendritic cell of this result to the wild type mouse origin is CpG. Although ODN was answered and IL-12 were produced, it sets to the dendritic cell of the TLR9 knockout-mouse origin, and it is CpG. ODN did not guide production of IL-12.

[0056] It cultivates by RPMI1640 culture medium which added 10% of foetal calf serum containing a 10 ng/above-mentioned ml mouse granulocyte macrophage colony-stimulating factor (product made from Peprotech). In the dendritic cell collected on the 6th, receive CD40, CD80, CD86, and the MHC class II. Each biotin-ized antibody dyes and it is made to develop in the streptoavidin which carried out the indicator by the phycoerythrin (phycoerythrin:PE; product made from fur MINJIEN). Cel QUEST software (product made from BEKUTONDIKKINSON) analyzed these cells by the fluorescence-activated-cell-sortor caliber (FACS Calibur) (drawing 8). From this result to CpG Although the manifestation of CD40, CD80, CD86, and the MHC class II was promoted in the dendritic cell front face of the wild type mouse origin when stimulated by ODN, in the dendritic cell front face of the TLR9 knockout-mouse origin, it is CpG. The manifestation of these molecules was not promoted by the response to ODN (drawing 8). In the stimulus by LPS, the response with the same said [the dendritic cell of the wild type mouse origin] of the dendritic cell of the TLR9 knockout-mouse origin was seen. The above result to TLR9 is CpG. It turned out that it is an acceptor indispensable to the cell response of ODN.

[0057] Example 5 (activation of NF-kappa B and JNK by the response to CpG ODN of the macrophage of the TLR9 knockout-mouse origin, and IRAK)

The signal of TLR activating IRAK which are a serine / threonine kinase through MyD88 which is an adapter molecule, and activating a MAP kinase and NF-kappa B subsequently is known (Immunity 11, 115-122, 1999). Then, CpG ODN investigated whether this intracellular signaling molecule would be activated. About the peritoneal macrophage (1x106cells) of the wild type mouse prepared according to the example 3, and TLR9 knockout mouse, it is CpG of 1.0microM. The time amount stimulus was carried out, nucleoprotein was extracted from the macrophage of each mouse, it incubated together with the specific probe including the DNA bonding site of NF-kappa B shown in drawing 9 by ODN or LPS of 1.0microg [/ml] Salmonella Minnesota Re-595, electrophoresis was performed, and it visualized with autoradiography (drawing 9 R> 9).

[0058] From this result to CpG When stimulated by ODN, by the macrophage of the TLR9 knockout-mouse origin, the DNA avidity of NF-kappa B did not increase to the DNA avidity of NF-kappa B increasing in the macrophage of the wild type mouse origin. Activation of the NF-kappa B as what stimulated the macrophage of the wild type mouse origin by LPS with what [same] stimulated the macrophage of the TLR9 knockout-mouse origin by LPS was seen. From the above result to CpG It turns out that the activity of NF-kappa B by induction of ODN is specifically missing in the macrophage of the TLR9 knockout-mouse origin. In addition, the arrow head in drawing shows the location of the composite of NF-kappa B and a specific probe, and Yato shows the location of only a specific probe.

[0059] The time amount shown by drawing 10 or drawing 11 like the above, CpG The macrophage of the wild type mouse stimulated by ODN or LPS, and TLR9 knockout mouse Dissolution buffer solution (it NaCl(s) 1.0% of triton X-100 and 137mM by the last concentration) Tris of 20mM(s) - EDTA of HCl and 5mM, 10% of glycerol, PMSF of 1mM, the 20micro ag [/ml] aprotinin, 20microg [/ml] leupeptin, The buffer solution containing beta-glycerophosphoric acid of Na3VO4 of 1mM, and 10mM(s); It dissolves in pH8.0. Immunoprecipitation of this cell melt is carried out by the anti-JNK antibody (made in Santa Cruz), or the anti-IRAK antibody (wood primeval national-chemical-laborator incorporated company make). Like a reference (Immunity 11, 115-122, 1999) publication In vitro kinase assay was performed and the JNK activity which made the substrate GST-c-Jun dissolution protein (GST-c-Jun), and the activity of IRAK were measured (drawing 10 , upper case;GST-c-Jun in 11, Auto).

[0060] Moreover, SDS-polyacrylamide gel electrophoresis was made to separate, the above-mentioned cell melt was moved to the nitrocellulose membrane, the blot of this film was carried out by the anti-JNK antibody (made in Santa Cruz), or the anti-IRAK antibody (product made from Transduction Laboratories), and it visualized using en HANSUDO chemiluminescence equipment (product made from DEYUPONTO) (drawing 10 , the lower berth in 11; WB). From the above result

to CpG Although ODN activated JNK and IRAK of a macrophage of the wild type mouse origin, in the macrophage of the TLR9 knockout-mouse origin, it turned out that it is not activated at all (drawing 10 , 11). Therefore, CpG It turned out that it depends for the signal transduction through ODN on TLR9.

[0061]

[Effect of the Invention] Although the bacteria origin DNA containing the CpG motif which is not methylated activated immunocyte very much and the response of Th1 was guided, the acceptor which recognizes the bacteria origin DNA was not known. Since the acceptor of an oligonucleotide including the non-methylating CpG array of bacterial DNA became clear by this invention, if the member receptor protein TLR9 of the TLR family which recognizes specifically the bacterial DNA which has a non-methylating CpG array, the gene DNA which carries out the code of it can be used for a diagnosis of a bacterially caused disease etc., and a therapy and a TLR9 knock-out animal is used, it will become possible [clarifying the operation mechanism in the molecular level of the bacteria origin DNA].

[0062]

[Layout Table]

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Leu Arg Gln Arg Leu Cys Arg Gln Ser Val 980 985 990 Leu Phe Trp

Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln 995 1000 1005 Leu Ser Thr AlaLeu ThrArg Asp Asn Arg His Phe Tyr Asn Gln Asn 1010 1015 1020 Phe Cys ArgGly Pro Thr Ala Glu 1025 1030 <210> 5 <211> 20<212> DNA <213> Artificial Sequence<220> <223> Descriptionof Artificial Sequence:CpG ODN <400> 5 tccatgacgt tcctgatgct 20

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the genetic map of TLR9 knockout mouse of this invention, and a wild type mouse.
[Drawing 2] It is drawing showing the result of the Southern blot analysis of TLR9 knockout mouse of this invention.
[Drawing 3] It is drawing showing the result of the Northern blot analysis in the spleen cell of TLR9 knockout mouse of this invention.
[Drawing 4] It is drawing showing the comparison result of the amino acid sequence of TLR9 knockout mouse of this invention, and a wild type mouse.
[Drawing 5] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of TNFalpha by ODN, PGN, or LPS induction, IL-6, or the amount of production of IL12.
[Drawing 6] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of the cell proliferation response by ODN or LPS induction.
[Drawing 7] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of the amount of production of IL-12 by ODN or LPS induction.
[Drawing 8] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of the amount of manifestations of the CD40, CD80, CD86, and the MHC class II by ODN or LPS induction.
[Drawing 9] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of activation of NF-kappa B by ODN or LPS induction.
[Drawing 10] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of the activation of JNK by ODN or LPS induction.
[Drawing 11] CpG in TLR9 knockout mouse and wild type mouse of this invention It is drawing showing the result of activation of ODN or IRAK by LPS induction.

[Translation done.]

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(54)【発明の名称】細菌ＤＮＡを特異的に認識する受容体タンパク質

(57)【要約】
【課題】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質や、それをコードする遺伝子ＤＮＡや、細菌性伝染病に対する宿主免疫細胞の応答性を調べる上で有用な実験モデル動物を提供すること。
【解決手段】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡを、ＢＬＡＳＴサーチによりスクリーニングし、各種ＴＬＲと高い相似性を有する多くのＥＳＴクローンをスクリーニングし、これらをプローブにして、マウス・マクロファージｃＤＮＡライブラリーから完全長ｃＤＮＡを単離し、ｃＤＮＡの塩基配列を解析してＬＲＲ及びＴＩＲ領域などの保存領域が存在するＴＬＲ９であることを確認した後、ノックアウトマウスを作製し、ＴＬＲ９が細菌ＤＮＡの非メチル化ＣｐＧ配列を含むオリゴヌクレオチドの受容体タンパク質であることを確認した。

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【特許請求の範囲】

【請求項１】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ。

【請求項２】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質が、以下の(ａ)又は(ｂ)のタンパク質であることを特徴とする請求項１記載のＤＮＡ。

(ａ)配列番号２に示されるアミノ酸配列からなるタンパク質

(ｂ)配列番号２に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなり、かつ非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質

【請求項３】　配列番号１に示される塩基配列又はその相補的配列並びにこれらの配列の一部または全部を含むことを特徴とする請求項１記載のＤＮＡ。

【請求項４】　請求項３記載の遺伝子を構成するＤＮＡとストリンジェントな条件下でハイブリダイズすることを特徴とする請求項１記載のＤＮＡ。

【請求項５】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質が、以下の(ａ)又は(ｂ)のタンパク質であることを特徴とする請求項１記載のＤＮＡ。

(ａ)配列番号４に示されるアミノ酸配列からなるタンパク質

(ｂ)配列番号４に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなり、かつ非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質

【請求項６】　配列番号３に示される塩基配列又はその相補的配列並びにこれらの配列の一部または全部を含むことを特徴とする請求項１記載のＤＮＡ。

【請求項７】　請求項６記載の遺伝子を構成するＤＮＡとストリンジェントな条件下でハイブリダイズすることを特徴とする請求項１記載のＤＮＡ。

【請求項８】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質。

【請求項９】　配列番号２に示されるアミノ酸配列からなることを特徴とする請求項８記載のタンパク質。

【請求項１０】　配列番号２に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなることを特徴とする請求項８記載のタンパク質。

【請求項１１】　配列番号４に示されるアミノ酸配列からなることを特徴とする請求項８記載のタンパク質。

【請求項１２】　配列番号４に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなることを特徴とする請求項８記載のタンパク質。

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【請求項１３】　請求項８～１２のいずれか記載のタンパク質と、マーカータンパク質及び／又はペプチドタグとを結合させた融合タンパク質。

【請求項１４】　請求項８～１２のいずれか記載のタンパク質と特異的に結合する抗体。

【請求項１５】　抗体がモノクローナル抗体であることを特徴とする請求項１４記載の抗体。

【請求項１６】　請求項８～１２のいずれか記載のタンパク質を発現することができる発現系を含んでなる宿主細胞。

【請求項１７】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現することを特徴とする非ヒト動物。

【請求項１８】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損したことを特徴とする非ヒト動物。

【請求項１９】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して不反応性であることを特徴とする請求項１８記載の非ヒト動物。

【請求項２０】　齧歯目動物が、マウスであることを特徴とする請求項１７～１９のいずれか記載の非ヒト動物。

【請求項２１】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した細胞に、請求項１～７のいずれか記載のＤＮＡを導入することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質を発現する細胞の調製方法。

【請求項２２】　請求項２１記載の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞の調製方法により得られることを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞。

【請求項２３】　被検物質の存在下、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現している細胞をインビトロで培養し、ＴＬＲ９活性を測定・評価することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法。

【請求項２４】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物に被検物質を投与し、該非ヒト動物から得られるマクロファージ又は脾臓細胞のＴＬＲ９活性を測定・評価することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法。

【請求項２５】　非メチル化ＣｐＧ配列を有する細菌Ｄ

ＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現した非ヒト動物に被検物質を投与し、該非ヒト動物から得られるマクロファージ又は脾臓細胞のＴＬＲ９活性を測定・評価することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法。

【請求項２６】　非ヒト動物が、マウスであることを特徴とする請求項２４又は２５記載の非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質のアゴニスト又はアンタゴニストのスクリーニング方法。

【請求項２７】　請求項２３～２６のいずれか記載の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法により得られる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニスト。

【請求項２８】　非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の全部又はその一部を有効成分として含有する医薬組成物。

【請求項２９】　請求項２７記載のアゴニスト又はアンタゴニストを有効成分として含有する医薬組成物。

【請求項３０】　検体中の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡと、請求項３記載のＤＮＡとの塩基配列を比較することができる、請求項３記載のＤＮＡを含むことを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ配列の欠失、置換及び／又は付加に関連する疾病の診断キット。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質、該受容体タンパク質の遺伝子及びそれらの利用に関する。

【０００２】

【従来の技術】トール（Ｔｏｌｌ）遺伝子は、ショウジョウバエの胚発生中の背腹軸の決定（Cell 52, 269-279, 1988、Annu. Rev. Cell Dev. Biol. 12, 393-416, 1996）、また成体における抗真菌性免疫応答に必要であることが知られている（Cell 86,973-983, 1996）。かかるＴｏｌｌは、細胞外領域にロイシンリッチリピート（ＬＲＲ）を有するＩ型膜貫通受容体であり、この細胞質内領域は、哺乳類インターロイキン－１受容体（ＩＬ－１Ｒ）の細胞質内領域と相同性が高いことが明らかとなっている（Nature 351, 355-356, 1991、Annu. Rev. Cell Dev. Biol. 12, 393-416, 1996、J. Leukoc. Biol. 63, 650-657, 1998）。

【０００３】近年、Ｔｏｌｌ様受容体（ＴＬＲ）と呼ばれるＴｏｌｌの哺乳類のホモログが同定され、ＴＬＲ２やＴＬＲ４など現在までに６つのファミリーが報告されている（Nature 388, 394-397, 1997、Proc. Natl. Acad. Sci. USA 95, 588-593, 1998、Blood 91, 4020-4027, 1998、Gene 231, 59-65, 1999）。このＴＬＲファミリーは、上記ＩＬ－１Ｒと同様にアダプタータンパク質であるＭｙＤ８８を介し、ＩＬ－１Ｒ結合キナーゼ（ＩＲＡＫ）をリクルートし、ＴＲＡＦ６を活性化し、下流のＮＦ－κＢを活性化することが知られている（J. Exp. Med. 187, 2097-2101, 1998、Mol. Cell 2, 253-258, 1998、Immunity 11, 115-122, 1999）。また、哺乳類におけるＴＬＲファミリーの役割は、細菌の共通構造を認識するパターン認識受容体（ＰＲＲ：pattern recognition receptor）として、先天的な免疫認識に関わっているとも考えられている（Cell 91, 295-298, 1997）。

【０００４】上記ＰＲＲにより認識される病原体会合分子パターン（ＰＡＭＰ：pathogen-associated molecular pattern）の一つは、グラム陰性菌の外膜の主成分であるリポ多糖（ＬＰＳ）であって（Cell 91, 295-298, 1997）、かかるＬＰＳが宿主細胞を刺激して宿主細胞にＴＮＦα、ＩＬ－１及びＩＬ－６等の各種炎症性サイトカインを産生させること（Adv. Immunol. 28, 293-450, 1979、Annu. Rev. Immunol. 13, 437-457, 1995）や、ＬＰＳ結合タンパク質（ＬＢＰ：LPS-bindingprotein）により捕獲されたＬＰＳが細胞表面上のＣＤ１４に引き渡されることが知られている（Science 249, 1431-1433, 1990、Annu. Rev. Immunol. 13, 437-457, 1995）。本発明者らは、ＴＬＲ４のノックアウトマウスを作製し、ＴＬＲ４ノックアウトウスが上記グラム陰性菌の外膜の主成分であるＬＰＳに不応答性であること（J. Immunol. 162, 3749-3752, 1999）や、ＴＬＲ２ノックアウトマウスを作製し、ＴＬＲ２ノックアウトマウスのマクロファージがグラム陽性菌細胞壁やその構成成分であるペプチドグリカンに対する反応性が低下すること（Immunity, 11, 443-451, 1999）を報告している。

【０００５】他方、細菌ＤＮＡ（バクテリア由来ＤＮＡ）や非メチル化ＣｐＧ配列を含むオリゴヌクレオチドが、マウス及びヒトの免疫細胞を刺激すること(Trends Microbiol. 4, 73-76, 1996、Trends Microbiol. 6, 496-500, 1998)や、ＩＬ－１２及びＩＦＮγの放出に支配されるＴヘルパー１細胞（Ｔｈ１）様炎症性応答を刺激すること(EMBO J. 18, 6973-6982, 1999、J. Immunol. 161, 3042-3049, 1998、Proc. Natl. Acad. Sci. USA 96, 9305-9310, 1999)から、ＣｐＧ配列を含むオリゴヌクレオチドは、癌、アレルギー及び伝染病のワクチンを含むワクチン戦略のアジュバントとしての使用可能性が提唱されている(Adv. Immunol. 73, 329-368, 1999、Curr. Opin. Immunol. 12, 35-43, 2000、Immunity 11, 1

23-129, 1999)．このように臨床実用において効果が期待されるにも関わらず、非メチル化ＣｐＧ配列を含む細菌ＤＮＡが免疫細胞を活性化する分子メカニズムはよくわかっていない。

【０００６】

【発明が解決しようとする課題】上記のように、メチル化されていないＣｐＧモチーフを含有するバクテリア由来ＤＮＡは免疫細胞を非常に活性化し、Ｔｈ１の応答を誘導するが、その分子レベルでの活動はあまり理解されていない．本発明の課題は、細菌ＤＮＡの非メチル化ＣｐＧ配列を含むオリゴヌクレオチドの分子レベルでの作用を明らかにすることができる、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識するＴＬＲファミリーのメンバー受容体タンパク質ＴＬＲ９や、それをコードするＤＮＡや、細菌性伝染病に対する宿主免疫細胞の応答性を調べる上で有用な実験モデル動物を提供することにある。

【０００７】

【課題を解決するための手段】細菌の共通構造を認識するパターン認識受容体として、先天的な免疫認識に関わっている哺乳類におけるＴＬＲファミリーは、現在までに６つのメンバー（ＴＬＲ１－６）が公表されており(Nature 388, 394-397, 1997、 Proc. Natl. Acad. Sci. USA, 95, 588-593, 1998、Gene 231, 59-65, 1999)、ＴＬＲ７及びＴＬＲ８の新たな２つのメンバーがGenBankに登録されている(登録番号ＡＦ２４０４６７及びＡＦ２４６９７１)。また、ＴＬＲ９についても完全長ｃＤＮＡが見い出されGenBankに登録されている(登録番号ＡＦ２４５７０４)が、その機能については知られていなかった。

【０００８】本発明者らは、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識するＴＬＲファミリーのメンバー受容体タンパク質をコードするＤＮＡを、ＢＬＡＳＴサーチによりスクリーニングし、既に同定されている各種ＴＬＲと高い相似性を有する多くのシークエンス・タグ（ＥＳＴ）クローンをスクリーニングし、これらの遺伝子フラグメントをプローブにして、マウス･マクロファージｃＤＮＡライブラリーから完全な長さを有するｃＤＮＡを単離し、これを用いてヒトｃＤＮＡも単離した．次に、これらｃＤＮＡの塩基配列を解析し、このＴＬＲファミリーにＬＲＲ及びＴＩＲ領域などの保存領域が存在するＴＬＲ９であることを確認した．そこで、このＴＬＲ９ノックアウトマウスを作製し、ＴＬＲ９が細菌ＤＮＡの非メチル化ＣｐＧ配列を含むオリゴヌクレオチドの受容体タンパク質であることを明らかにし、本発明を完成するに至った。

【０００９】すなわち本発明は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ（請求項１）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質が、以下の(ａ)又は(ｂ)のタンパク質であることを特徴とする請求項１記載のＤＮＡ(ａ)配列番号２に示されるアミノ酸配列からなるタンパク質(ｂ)配列番号２に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなり、かつ非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質（請求項２）や、配列番号１に示される塩基配列又はその相補的配列並びにこれらの配列の一部または全部を含むことを特徴とする請求項１記載のＤＮＡ（請求項３）や、請求項３記載の遺伝子を構成するＤＮＡとストリンジェントな条件下でハイブリダイズすることを特徴とする請求項１記載のＤＮＡ（請求項４）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質が、以下の(ａ)又は(ｂ)のタンパク質であることを特徴とする請求項１記載のＤＮＡ(ａ)配列番号４に示されるアミノ酸配列からなるタンパク質(ｂ)配列番号４に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなり、かつ非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質（請求項５）や、配列番号３に示される塩基配列又はその相補的配列並びにこれらの配列の一部または全部を含むことを特徴とする請求項１記載のＤＮＡ（請求項６）や、請求項６記載の遺伝子を構成するＤＮＡとストリンジェントな条件下でハイブリダイズすることを特徴とする請求項１記載のＤＮＡ（請求項７）に関する。

【００１０】また本発明は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質（請求項８）や、配列番号２に示されるアミノ酸配列からなることを特徴とする請求項８記載のタンパク質（請求項９）や、配列番号２に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなることを特徴とする請求項８記載のタンパク質（請求項１０）や、配列番号４に示されるアミノ酸配列からなることを特徴とする請求項８記載のタンパク質（請求項１１）や、配列番号４に示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなることを特徴とする請求項８記載のタンパク質（請求項１２）に関する。

【００１１】また本発明は、請求項８～１２のいずれか記載のタンパク質と、マーカータンパク質及び／又はペプチドタグとを結合させた融合タンパク質（請求項１３）や、請求項８～１２のいずれか記載のタンパク質と特異的に結合する抗体（請求項１４）や、抗体がモノクローナル抗体であることを特徴とする請求項１４記載の抗体（請求項１５）や、請求項８～１２のいずれか記載のタンパク質を発現することができる発現系を含んでなる宿主細胞（請求項１６）に関する。

【００１２】また本発明は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現することを特徴とする非ヒト動物（請求項１７）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損したことを特徴とする非ヒト動物（請求項１８）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して不反応性であることを特徴とする請求項１８記載の非ヒト動物（請求項１９）や、齧歯目動物が、マウスであることを特徴とする請求項１７～１９のいずれか記載の非ヒト動物（請求項２０）に関する。

【００１３】また本発明は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した細胞に、請求項１～７のいずれか記載のＤＮＡを導入することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質を発現する細胞の調製方法（請求項２１）や、請求項２１記載の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞の調製方法により得られることを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞（請求項２２）に関する。

【００１４】また本発明は、被検物質の存在下、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現している細胞をインビトロで培養し、ＴＬＲ９活性を測定・評価することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法（請求項２３）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物に被検物質を投与し、該非ヒト動物から得られるマクロファージ又は脾臓細胞のＴＬＲ９活性を測定・評価することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法（請求項２４）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現した非ヒト動物に被検物質を投与し、該非ヒト動物から得られるマクロファージ又は脾臓細胞のＴＬＲ９活性を測定・評価することを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法（請求項２５）や、非ヒト動物が、マウスであることを特徴とする請求項２４又は２５記載の非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質のアゴニスト又はアンタゴニストのスクリーニング方法（請求項２６）に関する。

【００１５】また本発明は、請求項２３～２６のいずれか記載の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法により得られる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニスト（請求項２７）や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の全部又はその一部を有効成分として含有する医薬組成物（請求項２８）や、請求項２７記載のアゴニスト又はアンタゴニストを有効成分として含有する医薬組成物（請求項２９）や、検体中の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡと、請求項３記載のＤＮＡとの塩基配列を比較することができる、請求項３記載のＤＮＡを含むことを特徴とする非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ配列の欠失、置換及び／又は付加に関連する疾病の診断キット（請求項３０）に関する。

【００１６】

【発明の実施の形態】本発明における非メチル化ＣｐＧ配列を有する細菌ＤＮＡとしては、Ｔ細胞、Ｂ細胞、抗原提示細胞等の免疫細胞を活性化し、免疫応答を誘導することができる、メチル化されていないＣｐＧモチーフを有するオリゴデオキシヌクレオチド（ＯＤＮ）等のバクテリアに由来するＤＮＡであればどのようなものでもよく、エセリシア・コリ、クレブシェラ・ニューモニエ、シュードモナス・アエルギノサ、サルモネラ・チフィムリウム、セラチア・マルセッセンス、フレクスナー赤痢菌、ビブリオ・コレレェ、サルモネラ・ミネソタ、ポルフィロモナス・ジンジバリス、スタフィロコッカス・アウレウス、コリネバクテリウム・ジフテリア、ノカルジア・コエリアカ、ストレプトコッカス・ニューモニアなどのバクテリア由来のＤＮＡを具体的に挙げることができる。

【００１７】かかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質としては、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識することができるタンパク質であれば特に制限されるものではなく、例えば、配列表の配列番号２で示されるヒト由来のＴＬＲ９や、配列番号２で示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなり、かつ上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識することができるタンパク質や、これらの組換えタンパク質を具体的に挙げることができる。かかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質は、そのＤＮＡ配列情報等に基づき公知の方法で調製することができる。

【００１８】また、本発明の非メチル化ＣｐＧ配列を有

する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡとしては、配列表の配列番号２で示されるヒト由来のＴＬＲ９をコードするＤＮＡ、例えば配列番号１で示されるものや、配列番号２で示されるアミノ酸配列において、１若しくは数個のアミノ酸が欠失、置換若しくは付加されたアミノ酸配列からなり、かつ上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識することができるタンパク質をコードするＤＮＡや、これらＤＮＡとストリンジェントな条件下でハイブリダイズし、かつ上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識することができるタンパク質をコードするＤＮＡも包含され、これらはそのＤＮＡ配列情報等に基づき、例えばマウス由来のＴＬＲ９においてはマウスＲＡＷ２６４．７ｃＤＮＡライブラリーや１２９／ＳｖＪマウス遺伝子ライブラリーなどから公知の方法により調製することができる。

【００１９】また、配列番号１に示される塩基配列又はその相補的配列並びにこれらの配列の一部又は全部をプローブとして、マウス由来のＤＮＡライブラリーに対してストリンジェントな条件下でハイブリダイゼーションを行ない、該プローブにハイブリダイズするＤＮＡを単離することにより、受容体タンパク質ＴＬＲ９と同効な目的とする免疫誘導非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡを得ることもできる。かかるＤＮＡを取得するためのハイブリダイゼーションの条件としては、例えば、４２℃でのハイブリダイゼーション、及び１×ＳＳＣ、０．１％のＳＤＳを含む緩衝液による４２℃での洗浄処理を挙げることができ、６５℃でのハイブリダイゼーション、及び０．１×ＳＳＣ，０．１％のＳＤＳを含む緩衝液による６５℃での洗浄処理をより好ましく挙げることができる。なお、ハイブリダイゼーションのストリンジェンシーに影響を与える要素としては、上記温度条件以外に種々の要素があり、当業者であれば、種々の要素を適宜組み合わせて、上記例示したハイブリダイゼーションのストリンジェンシーと同等のストリンジェンシーを実現することが可能である。

【００２０】本発明の融合タンパク質とは、マウス、ヒト等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に、マーカータンパク質及び／又はペプチドタグを結合させたものをいい、マーカータンパク質としては、従来知られているマーカータンパク質であればどのようなものでもよく、例えば、アルカリフォスファターゼ、抗体のＦｃ領域、ＨＲＰ、ＧＦＰなどを具体的に挙げることができ、また本発明におけるペプチドタグとしては、Ｍｙｃタグ、Ｈｉｓタグ、ＦＬＡＧタグ、ＧＳＴタグなどの従来知られているペプチドタグを具体的に例示することができる。かかる融合タンパク質は、常法により作製することができ、Ｎｉ－ＮＴＡとＨｉｓタグの親和性を利用した非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の精製や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の検出や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に対する抗体の定量や、その他当該分野の研究用試薬としても有用である。

【００２１】本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に特異的に結合する抗体としては、モノクローナル抗体、ポリクローナル抗体、キメラ抗体、一本鎖抗体、ヒト化抗体等の免疫特異的な抗体を具体的に挙げることができ、これらは上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を抗原として用いて常法により作製することができるが、その中でもモノクローナル抗体がその特異性の点でより好ましい。かかるモノクローナル抗体等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に特異的に結合する抗体は、例えば、ＴＬＲ９の変異又は欠失に起因する疾病の診断やＴＬＲ９の制御分子機構を明らかにする上で有用である。

【００２２】非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に対する抗体は、慣用のプロトコールを用いて、動物（好ましくはヒト以外）に該非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質若しくはエピトープを含む断片、又は該タンパク質を膜表面に発現した細胞を投与することにより産生され、例えばモノクローナル抗体の調製には、連続細胞系の培養物により産生される抗体をもたらす、ハイブリドーマ法（Nature 256, 495-497, 1975）、トリオーマ法、ヒトＢ細胞ハイブリドーマ法（ImmunologyToday 4, 72, 1983）及びＥＢＶ－ハイブリドーマ法（MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp.77-96, Alan R.Liss, Inc., 1985）など任意の方法を用いることができる。以下に非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質として、マウス由来のＴＬＲ９を例に挙げてマウス由来のＴＬＲ９に対して特異的に結合するモノクローナル抗体、すなわち抗ｍＴＬＲ９モノクローナル抗体の作製方法を説明する。

【００２３】上記抗ｍＴＬＲ９モノクローナル抗体は、抗ｍＴＬＲ９モノクローナル抗体産生ハイブリドーマをインビボ又はインビトロで常法により培養することにより生産することができる。例えば、インビボ系においては、齧歯動物、好ましくはマウス又はラットの腹腔内で培養することにより、またインビトロ系においては、動物細胞培養用培地で培養することにより得ることができる。インビトロ系でハイブリドーマを培養するための培地としては、ストレプトマイシンやペニシリン等の抗生物質を含むＲＰＭＩ１６４０又はＭＥＭ等の細胞培養培地を例示することができる。

【００２４】抗ｍＴＬＲ９モノクローナル抗体産生ハイブリドーマは、例えば、マウス等から得られた受容体タンパク質ＴＬＲ９を用いてＢＡＬＢ／ｃマウスを免役し、免疫されたマウスの脾臓細胞とマウスＮＳ－１細胞（ＡＴＣＣ　ＴＩＢ－１８）とを、常法により細胞融合させ、免疫蛍光染色パターンによりスクリーニングすることにより、抗ｍＴＬＲ９モノクローナル抗体産生ハイブリドーマを作出することができる。また、かかるモノクローナル抗体の分離・精製方法としては、タンパク質の精製に一般的に用いられる方法であればどのような方法でもよく、アフィニティークロマトグラフィー等の液体クロマトグラフィーを具体的に例示することができる。

【００２５】また、本発明の上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に対する一本鎖抗体をつくるためには、一本鎖抗体の調製法（米国特許第4,946,778号）を適用することができる。また、ヒト化抗体を発現させるために、トランスジェニックマウス又は他の哺乳動物等を利用したり、上記抗体を用いて、その非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現するクローンを単離・同定したり、アフィニティークロマトグラフィーでそのポリペプチドを精製することもできる。非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に対する抗体は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の分子機構を明らかにする上で有用である。

【００２６】また上記抗ｍＴＬＲ９モノクローナル抗体等の抗体に、例えば、ＦＩＴＣ（フルオレセインイソシアネート）又はテトラメチルローダミンイソシアネート等の蛍光物質や、^{125}Ｉ、^{32}Ｐ、^{35}Ｓ又は^{3}Ｈ等のラジオアイソトープや、アルカリホスファターゼ、ペルオキシダーゼ、β－ガラクトシダーゼ又はフィコエリトリン等の酵素で標識したものや、グリーン蛍光タンパク質（ＧＦＰ）等の蛍光発光タンパク質などを融合させた融合タンパク質を用いることによって、上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の機能解析を行うことができる。また免疫学的測定方法としては、ＲＩＡ法、ＥＬＩＳＡ法、蛍光抗体法、プラーク法、スポット法、血球凝集反応法、オクタロニー法等の方法を挙げることができる。

【００２７】本発明はまた、上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現することができる発現系を含んでなる宿主細胞に関する。かかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子の宿主細胞への導入は、Davisら（BASIC METHODS IN MOLECULAR BIOLOGY, 1986）及びSambrookら（MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold SpringHarbor, N.Y., 1989）などの多くの標準的な実験室マニュアルに記載される方法、例えば、リン酸カルシウムトランスフェクション、ＤＥＡＥ－デキストラン媒介トランスフェクション、トランスベクション(transvection)、マイクロインジェクション、カチオン性脂質媒介トランスフェクション、エレクトロポレーション、形質導入、スクレープローディング（scrape loading）、弾丸導入(ballistic introduction)、感染等により行うことができる。そして、宿主細胞としては、大腸菌、ストレプトミセス、枯草菌、ストレプトコッカス、スタフィロコッカス等の細菌原核細胞や、酵母、アスペルギルス等の真菌細胞や、ドロソフィラＳ２、スポドプテラＳｆ９等の昆虫細胞や、Ｌ細胞、ＣＨＯ細胞、ＣＯＳ細胞、ＨｅＬａ細胞、Ｃ１２７細胞、ＢＡＬＢ／ｃ３Ｔ３細胞（ジヒドロ葉酸レダクターゼやチミジンキナーゼなどを欠損した変異株を含む）、ＢＨＫ２１細胞、ＨＥＫ２９３細胞、Ｂｏｗｅｓメラノーマ細胞、卵母細胞等の動植物細胞などを挙げることができる。

【００２８】また、発現系としては、上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を宿主細胞内で発現させることができる発現系であればどのようなものでもよく、染色体、エピソーム及びウイルスに由来する発現系、例えば、細菌プラスミド由来、酵母プラスミド由来、ＳＶ４０のようなパポバウイルス、ワクシニアウイルス、アデノウイルス、鶏痘ウイルス、仮性狂犬病ウイルス、レトロウイルス由来のベクター、バクテリオファージ由来、トランスポゾン由来及びこれらの組合せに由来するベクター、例えば、コスミドやファージミドのようなプラスミドとバクテリオファージの遺伝的要素に由来するものを挙げることができる。これら発現系は、発現を起こさせるだけでなく、発現を調節する制御配列を含んでいてもよい。

【００２９】上記発現系を含んでなる宿主細胞やかかる細胞の細胞膜、またかかる細胞を培養して得られる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質は、後述するように本発明のスクリーニング方法に用いることができる。例えば、細胞膜を得る方法としては、F. Pietri-Rouxel (Eur. J. Biochem., 247, 1174-1179, 1997) らの方法などを用いることができ、また、かかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を細胞培養物から回収し精製するには、硫酸アンモニウムまたはエタノール沈殿、酸抽出、アニオンまたはカチオン交換クロマトグラフィー、ホスホセルロースクロマトグラフィー、疎水性相互作用クロマトグラフィー、アフィニティークロマトグラフィー、ハイドロキシアパタイトクロマトグラフィーおよびレクチンクロマトグラフィーを含めた公知の方法、好ましくは、高速液体クロマトグラフィーが用いられる。特に、アフィニティークロマトグラ

フィーに用いるカラムとしては、例えば、抗ＴＬＲ９モノクローナル抗体等の抗非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質抗体を結合させたカラムや、上記ＴＬＲ９等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に通常のペプチドタグを付加した場合は、このペプチドタグに親和性のある物質を結合したカラムを用いることにより、これらの非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を得ることができる。

【００３０】本発明において、上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現する非ヒト動物とは、野生型非ヒト動物に比べてかかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を大量に産生する非ヒト動物をいい、また、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物とは、染色体上の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子の一部若しくは全部が破壊・欠損・置換等の遺伝子変異により不活性化され、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する機能を失なった非ヒト動物をいう。そして、本発明における非ヒト動物としては、ウサギや、マウス、ラット等の齧歯目動物などの非ヒト動物を具体的に挙げることができるが、これらに限定されるものではない。

【００３１】また、本発明において非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して不反応性とは、細菌ＤＮＡによる刺激に対する生体又は生体を構成する細胞、組織若しくは器官の反応性が低下しているか、あるいはほぼ失われていることを意味する。したがって、本発明において非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して不反応性の非ヒト動物とは、細菌ＤＮＡによる刺激に対して、生体又は生体を構成する細胞、組織若しくは器官の反応性が低下しているか、あるいはほぼ失われているマウス、ラット、ウサギ等のヒト以外の動物をいう。また、細菌ＤＮＡによる刺激としては、細菌ＤＮＡを生体に投与するインビボでの刺激や、生体から分離された細胞に細菌ＤＮＡを接触させるインビトロでの刺激等を挙げることができ、具体的には、ＴＬＲ９ノックアウトマウス等のＴＬＲ９遺伝子機能が染色体上で欠損した非ヒト動物を挙げることができる。

【００３２】ところで、メンデルの法則に従い出生してくるホモ接合体非ヒト動物には、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質欠損型又は過剰発現型とその同腹の野生型とが含まれ、これらホモ接合体非ヒト動物における欠損型又は過剰発現型とその同腹の野生型を同時に用いることによって個体レベルで正確な比較実験をすることができることから、野生型の非ヒト動物、すなわち非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損又は過剰発現する非ヒト動物と同種の動物、さらには同腹の動物を、例えば下記に記載する本発明のスクリーニングに際して併用することが好ましい。かかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損又は過剰発現する非ヒト動物の作製方法を、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のノックアウトマウスやトランスジェニックマウスを例にとって以下説明する。

【００３３】例えば、ＴＬＲ９等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損したマウス、すなわち非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質ノックアウトマウスは、マウス遺伝子ライブラリーからＰＣＲ等の方法により得られた遺伝子断片を用いて、上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子をスクリーニングし、スクリーニングされた非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子をウイルスベクター等を用いてサブクローンし、ＤＮＡシーケンシングにより特定する。このクローンの非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子の全部又は一部をｐＭＣ１ネオ遺伝子カセット等に置換し、３′末端側にジフテリアトキシンＡフラグメント（ＤＴ－Ａ）遺伝子や単純ヘルペスウイルスのチミジンキナーゼ（ＨＳＶ－ｔｋ）遺伝子等の遺伝子を導入することによって、ターゲットベクターを作製する。

【００３４】この作製されたターゲティングベクターを線状化し、エレクトロポレーション（電気穿孔）法等によってＥＳ細胞に導入し、相同的組換えを行い、その相同的組換え体の中から、Ｇ４１８やガンシクロビア（ＧＡＮＣ）等の抗生物質により相同的組換えを起こしたＥＳ細胞を選択する。また、この選択されたＥＳ細胞が目的とする組換え体かどうかをサザンブロット法等により確認することが好ましい。その確認されたＥＳ細胞のクローンをマウスの胚盤胞中にマイクロインジェクションし、かかる胚盤胞を仮親のマウスに戻し、キメラマウスを作製する。このキメラマウスを野生型のマウスとインタークロスさせると、ヘテロ接合体マウスを得ることができ、また、このヘテロ接合体マウスをインタークロスさせることによって、本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質ノックアウトマウスを作製することができる。また、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識

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する受容体タンパク質ノックアウトマウスが生起しているかどうかを確認する方法としては、例えば、上記の方法により得られたマウスからＲＮＡを単離してノーザンブロット法等により調べたり、またこのマウスの発現をウエスタンブロット法等により調べる方法がある。

【００３５】また、作出されたＴＬＲ９ノックアウトマウスが非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して不応答性であることは、例えば、ＣｐＧ　ＯＤＮをＴＬＲ９ノックアウトマウスのマクロファージ、単核細胞、樹状細胞などの免疫細胞にインビトロ又はインビボで接触せしめ、かかる細胞におけるＴＮＦ－α、ＩＬ－６、ＩＬ－１２、ＩＦＮ－γ等の産生量や、脾臓Ｂ細胞の増殖応答や、脾臓Ｂ細胞表面でのＣＤ４０、ＣＤ８０、ＣＤ８６、ＭＨＣクラスII等の抗原の発現量や、ＮＦ－κＢ、ＪＮＫ、ＩＲＡＫ等のＴＬＲ９のシグナル伝達経路における分子の活性化を測定することにより確認することができる。そして、本発明のＴＬＲ９ノックアウトマウスは、非メチル化ＣｐＧ配列を有する細菌ＤＮＡ等の作用機序の解明や細菌感染に対するワクチン開発に有用なモデルとすることができる。

【００３６】非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のトランスジェニックマウスは、ＴＬＲ９等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするｃＤＮＡにチキンβ－アクチン、マウスニューロフィラメント、ＳＶ４０等のプロモーター、及びラビットβ－グロビン、ＳＶ４０等のポリＡ又はイントロンを融合させて導入遺伝子を構築し、該導入遺伝子をマウス受精卵の前核にマイクロインジェクションし、得られた卵細胞を培養した後、仮親のマウスの輸卵管に移植し、その後被移植動物を飼育し、産まれた仔マウスから前記ｃＤＮＡを有する仔マウスを選択することによりかかるトランスジェニックマウスを創製することができる。また、ｃＤＮＡを有する仔マウスの選択は、マウスの尻尾等より粗ＤＮＡを抽出し、導入した非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子をプローブとするドットハイブリダイゼーション法や、特異的プライマーを用いたＰＣＲ法等により行うことができる。

【００３７】また、本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡの全部あるいは一部を用いると、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の欠失又は異常に起因する疾病等の遺伝子治療に有効な細胞を調製することができる。本発明におけるこれら細胞の調製方法としては、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した細胞に、上記本発明のＤＮＡの全部あるいは一部をトランスフェクション等により導入し、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞を得る方法を挙げることができ、特に、かかる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞としては、上記ＤＮＡ等が染色体にインテグレイトされ、ステイブルにＴＬＲ９活性を示す細胞を用いることが好ましい。

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【００３８】そしてまた、上記非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質とマーカータンパク質及び／又はペプチドタグとを結合させた融合非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に対する抗体、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現することができる発現系を含んでなる宿主細胞、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現する非ヒト動物、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞等を用いると、本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストや、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対する反応性の抑制物質又は促進物質をスクリーニングすることができる。これらのスクリーニングにより得られたものは、細菌感染症に対する抑制物質又は促進物質や、アレルギー性疾患若しくは癌に対する抑制剤、予防剤又は治療薬や、遺伝子治療等において副作用を抑制剤又は阻害剤や、ＴＬＲ９活性の欠失又は異常に起因する疾病等の診断・治療に有用な物質である可能性がある。

【００３９】上記ＴＬＲ９活性とは、非メチル化ＣｐＧ配列を有する細菌ＤＮＡと特異的に反応し、細胞内にシグナルを伝達する機能をいい、シグナル伝達機能としては、ＴＮＦ－α、ＩＬ－６、ＩＬ－１２、ＩＦＮ－γ等のサイトカインを産生する機能や、亜硝酸イオンを産生する機能や、細胞を増殖する機能や、細胞表面においてＣＤ４０、ＣＤ８０、ＣＤ８６、ＭＨＣクラスII等の抗原を発現する機能や、ＮＦ－κＢ、ＪＮＫ、ＩＲＡＫ等のＴＬＲ９のシグナル伝達経路における分子を活性化させる機能などを具体的に例示することができるが、これらに限定されるものではない。

【００４０】本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質のアゴニスト又はアンタゴニストのスクリーニング方法としては、被検物質の存在下、マクロファージ、脾臓細胞又は樹状細胞などの免疫細胞、非メチル化ＣｐＧ配列を有す

る細菌ＤＮＡを特異的に認識する受容体タンパク質を発現している細胞、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質を発現する細胞等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質を発現している細胞をインビトロで培養し、ＴＬＲ９活性を測定・評価する方法や、野生型非ヒト動物、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物、又は、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子が過剰発現した非ヒト動物に被検物質を投与し、該非ヒト動物から得られるマクロファージ、脾臓細胞、又は樹状細胞などの免疫細胞のＴＬＲ９活性を測定・評価する方法等を具体的に挙げることができる。

【００４１】また、マクロファージ活性又は脾臓細胞活性の程度を測定・評価するに際し、対照として野生型非ヒト動物、特に同腹の野生型非ヒト動物の測定値と比較・評価することが個体差によるバラツキをなくすることができるので好ましい。このことは、以下に示す非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対する反応性の抑制物質又は促進物質のスクリーニングにおいても同様である。

【００４２】また、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対する反応性の抑制物質又は促進物質のスクリーニング方法としては、被検物質と非メチル化ＣｐＧ配列を有する細菌ＤＮＡとの存在下、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質、又は該タンパク質を発現している細胞膜をインビトロでインキュベーションし、該タンパク質との反応性を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物から得られるマクロファージ又は脾臓細胞と被検物質とをあらかじめインビトロで接触せしめた後、該マクロファージ又は脾臓細胞を非メチル化ＣｐＧ配列を有する細菌ＤＮＡの存在下で培養し、該マクロファージ若しくは脾臓細胞のマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物から得られるマクロファージ又は脾臓細胞と非メチル化ＣｐＧ配列を有する細菌ＤＮＡとをあらかじめインビトロで接触せしめた後、該マクロファージ又は脾臓細胞を被検物質の存在下で培養し、該マクロファージ若しくは脾臓細胞のマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物にあらかじめ被検物質を投与した後、該非ヒト動物から得られるマクロファージ又は脾臓細胞を非メチル化ＣｐＧ配列を有する細菌ＤＮＡの存在下で培養し、該マクロファージ若しくは脾臓細胞のマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物にあらかじめ被検物質を投与した後、該非ヒト動物を細菌により感染させ、該非ヒト動物から得られるマクロファージ若しくは脾臓細胞のマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物をあらかじめ細菌により感染させた後、該非ヒト動物から得られるマクロファージ又は脾臓細胞を被検物質の存在下で培養し、該マクロファージ若しくは脾臓細胞のマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物をあらかじめ細菌により感染させた後、該非ヒト動物に被検物質を投与し、該非ヒト動物から得られるマクロファージ若しくは脾臓細胞のマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物にあらかじめ被検物質を投与した後、該非ヒト動物を細菌により感染させ、該非ヒト動物におけるマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法や、非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対して反応性を有するタンパク質をコードする遺伝子機能が染色体上で欠損した非ヒト動物をあらかじめ細菌により感染させた後、該非ヒト動物に被検物質を投与し、該非ヒト動物におけるマクロファージ活性又は脾臓細胞活性の程度を測定・評価する方法などを具体的に挙げることができる。また、これらのスクリーニング方法に用いる非メチル化ＣｐＧ配列を有する細菌ＤＮＡとしては、ＣｐＧ　ＯＤＮ（ＴＣＣ－ＡＴＧ－ＡＣＧ－ＴＴＣ－ＣＴＧ－ＡＴＧ－ＣＴ：配列番号５）を用いることが好ましいが、これに限定されるのもではない。

【００４３】本発明はまた、検体中の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ配列を、本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ配列と比較することからなる、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の活性又は発現と関連する疾病の診断に用いられる診断キットに関する。非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡの変異型の検出は、遺伝子に変異がある個体をＤＮＡレベルで見い出す

ことにより行うことができ、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の過少発現、過剰発現又は変異発現により生ずる疾病の診断に有効である。かかる検出に用いられる検体としては、被験者の細胞、例えば血液、尿、唾液、組織等の生検から得ることができるゲノムＤＮＡや、ＲＮＡ又はｃＤＮＡを具体的に挙げることができるがこれらに限定されるものではなく、かかる検体を使用する場合、ＰＣＲ等により増幅したものを用いることもできる。そして、塩基配列の欠失や挿入変異は、正常な遺伝子型と比較したときの増幅産物のサイズの変化により検出でき、また点突然変異は増幅ＤＮＡを標識非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子とハイブリダイズさせることで同定することができる。このように、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードする遺伝子の変異を検出することで、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の活性又は発現と関連する疾病の診断又は判定をすることができる。

【００４４】本発明はまた、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ又はＲＮＡのアンチセンス鎖の全部又は一部からなる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の活性又は発現と関連する疾患の診断用プローブ、及び当該プローブ及び／又は本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に特異的に結合する抗体を含有してなる非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の活性又は発現と関連する疾患の診断キットに関する。前記診断用プローブとしては、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ（ｃＤＮＡ）又はＲＮＡ（ｃＲＮＡ）のアンチセンス鎖の全部又は一部であり、プローブとして成立する程度の長さ（少なくとも２０ベース以上）を有するものであれば特に制限されるものではない。かかるプローブ及び／又は本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質に特異的に結合する抗体を細菌感染症等のような症状の疾患の診断薬の有効成分とするためには、プローブが分解されないような適当なバッファー類や滅菌水に溶解することが好ましい。また、これらの診断薬を用いた、免疫染色法（Dev.Biol. 170, 207-222, 1995、J. Neurobiol. 29, 1-17, 1996）や、In situハイブリダイゼーション法（J. Neurobiol. 29, 1-17, 1996）や、in situ PCR法等の方法により細菌感染症等のような症状の疾患を診断することもできる。

【００４５】本発明の医薬組成物としては、ＴＬＲ９等の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質の全部又はその一部や、上記受容体タンパク質のアゴニストやアンタゴニストを含むものであれば、どのようなものでもよい。具体的には、細菌感染症に対するワクチンや、癌に対するワクチンや、気管支喘息をはじめとするアレルギー疾患の治療薬や、アンチセンスオリゴヌクレオチドを用いた治療や遺伝子治療において障害となるＣｐＧモチーフの存在による副作用の克服剤・抑制剤・阻害剤などを挙げることができる。

【００４６】前記のように、本発明の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ配列の欠失、置換及び／又は付加に関連する疾病の診断キットとしては、ＴＬＲ９をコードするＤＮＡを含むものであればどのようなものでもよく、かかるＴＬＲ９をコードするＤＮＡと検体中の非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡとの塩基配列を比較することにより、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識する受容体タンパク質をコードするＤＮＡ配列の欠失、置換及び／又は付加に関連する疾病、例えば、癌、アレルギー、伝染病等の診断が可能となる。

【００４７】

【実施例】以下に、実施例を挙げてこの発明を更に具体的に説明するが、この発明の技術的範囲はこれら実施例により限定されるものではない。

実施例１（ＴＬＲ９のクローニング）

ヒトＴＬＲ４のＤＮＡ配列情報を用いて、GenBankをサーチした結果、相同性がきわめて高いマウスＥＳＴ（登録番号ＡＡ２７３７３１；マウス）を見い出した。このマウスＥＳＴのＰＣＲ増幅産物をプローブとして、マウスＲＡＷ２６４．７ｃＤＮＡライブラリーをスクリーニングし、完全なＴＬＲ９オープンリーディングフレームを含む配列番号３に示される完全長のｃＤＮＡクローンを単離した。このマウスＴＬＲ９のＤＮＡ配列情報を用いてGenBankをサーチし、高い相同性を有するヒトゲノム配列を見い出した。このヒトゲノム配列に基づいて、ｃＤＮＡ端部を増幅し、Ｕ９３７細胞（J. Immunol. 163, 5039-5048, 1999）から、配列番号１に示される塩基配列を有する完全長のヒトＴＬＲ９のｃＤＮＡを単離した。

【００４８】実施例２（ＴＬＲ９ノックアウトマウスの作製）

１２９／ＳｖＪマウス遺伝子ライブラリー（ストラタジーン社製）からＴＬＲ９ゲノムＤＮＡを単離し、pBluescript II SK(+)ベクター（ストラタジーン社製）中でサブクローンし、制限酵素マッピング及びＤＮＡ配列決定により特定した。ターゲッティングベクターは、ＬＲＲ（ロイシンリッチリピート）領域の一部分をコードする１．０ｋｂのフラグメントを、ネオマイシン耐性遺伝子

カセット（pMC1-neo；ストラタジーン社製）に置換し、負の選択マーカーとして単純ヘルペスウィルスチミジンキナーゼ（ＨＳＶ－ＴＫ）を挿入することにより構築した（図１）。このターゲッティングベクターを線状化し、胎生１４．１日目の胚幹細胞（ＥＳ細胞）にエレクトポレーションし、Ｇ４１８及びガンシクロビアに抵抗性を示す２９２個のクローンを選択し、ＰＣＲ法及びサザンブロット法により１４個のクローンをスクリーニングした。

【００４９】突然変異ＴＬＲ９対立遺伝子を含有していた３個の標的ＥＳクローンを、Ｃ５７ＢＬ／６マウスの胚盤胞中にマイクロインジェクションしキメラマウスを作製した。この雄のキメラマウスをＣ５７ＢＬ／６雌マウスと交配させ、ヘテロ接合体Ｆ１マウスを作製し、かかるヘテロ接合体Ｆ１マウスをインタークロスすることによってホモ接合体マウス（ＴＬＲ９ノックアウトマウス：$TLR9^{-/-}$）を得た（図２）。なお、ホモ接合体マウスの確認は、マウスの尾から抽出した各ゲノムＤＮＡをＳｃａＩでダイジェストし、図１に示すプローブを用いたサザンブロット法により行った。本発明のＴＬＲ９ノックアウトマウス（$TLR9^{-/-}$）はメンデルの法則に従い作製することができ、１２週目までは顕著な異常を示さなかった。

【００５０】突然変異によりＴＬＲ９遺伝子の不活性化が生起していることを確認するため、野生型マウス（＋／＋）及びＴＬＲ９ノックアウトマウス（－／－）の脾臓細胞から抽出した全ＲＮＡ（１０μｇ）を電気泳動にかけナイロン膜に移して、[^{32}P]で標識したＴＬＲ９のＣ－末端フラグメント若しくはＮ－末端フラグメント、又はβ－アクチン（β－ａｃｔｉｎ）に特異的なｃＤＮＡを用いてノーザンブロット分析を行った（図３）。これらの結果から、ＴＬＲ９ｍＲＮＡのＮ－末端フラグメントはＴＬＲ９ノックアウトマウスの脾臓細胞からは検出されなかった。また、Ｃ－末端フラグメントをプローブとした場合、変異マウス由来のＴｌｒ９の転写は野生型マウス由来のものとほぼ同じサイズのものが検出されたが、生産量においては少ないことがわかった。そこで、変異マウスから得られた脾臓細胞のｍＲＮＡを用いてＲＴ－ＰＣＲ法を行い、得られた生成物の配列分析を行った。この結果、転写されたＴｌｒ９遺伝子にはｎｅｏ遺伝子が含まれており、このｎｅｏの挿入によって、ＴＬＲ９のＮ－末端部位にストップコドンが出現し、変異マウスにおいて機能的なＴＬＲ９タンパク質が発現しないことがわかった（図４）。なお、ＴＬＲ９ノックアウトマウスのリンパ細胞をフローサイトメトリーで測定した結果、異常成分は見られなかった。

【００５１】実施例３（腹腔マクロファージの調製）
野生型マウス（ｗｉｌｄ－ｔｙｐｅ）及びＴＬＲ９ノックアウトマウス（$TLR9^{-/-}$）のそれぞれの腹腔内に４％のチオグリコール酸培地（ＤＩＦＣＯ社製）を２ｍｌずつ注入し、３日後に各マウスの腹腔内から腹膜滲出細胞を単離し、これらの細胞を１０％のウシ胎仔血清（ＧＩＢＣＯ社製）を添加したＲＰＭＩ１６４０培地（ＧＩＢＣＯ社製）中で３７℃にて２時間培養し、氷温のハンクス緩衝液（Hank's buffered salt solution：ＨＢＳＳ；ＧＩＢＣＯ社製）で洗浄することにより非付着細胞を取り除き、付着細胞を腹膜マクロファージとして以下の実験に使用した。

【００５２】実施例４（ＴＬＲ９ノックアウトマウスの非メチル化ＣｐＧ配列を有する細菌ＤＮＡに対する応答性）
最近、ＣｐＧ　ＯＤＮ（oligodeoxynucleotide）の応答性は、ＴＬＲを介するシグナル伝達経路の中のアダプタータンパク質であるＭｙＤ８８に依存していることが明らかになった。このＭｙＤ８８ノックアウトマウスはＣｐＧ　ＯＤＮに対して応答しないが、ＴＬＲ２ノックアウトマウスやＴＬＲ４ノックアウトマウスは正常にＣｐＧ　ＯＤＮに対して応答する。これらのことは、ＣｐＧ　ＯＤＮがＴＬＲ２及びＴＬＲ４以外のＴＬＲによって認識されることを示している。そこで、ＴＬＲ９ノックアウトマウスのＣｐＧ　ＯＤＮに対する応答性を調べてみた。まず、腹腔マクロファージにおける炎症性サイトカインの産生量を以下のように測定した。

【００５３】実施例３により調製した各腹膜マクロファージをＩＮＦγ（３０ｕｎｉｔ／ｍｌ）の存在下又は非存在下において、図５に示された各種濃度のＣｐＧ　ＯＤＮ（０．１又は１．０μＭ；TIB MOLBIOL社製；ＴＣＣ－ＡＴＧ－ＡＣＧ－ＴＴＣ－ＣＴＧ－ＡＴＧ－ＣＴ）、ＰＧＮ（１０μｇ／ｍｌ；Sigma and Fluka社製；スタフィロコッカス・アウレウス由来）、ＬＰＳ（１．０μｇ／ｍｌ；Sigma社製；サルモネラ・ミネソタＲｅ－５９５由来）といっしょに２４時間培養した。培養後、培養上清中のＴＮＦα、ＩＬ－６及びＩＬ－１２　ｐ４０の各濃度をＥＬＩＳＡ法により測定した。この結果を図５に示す。これらの結果から、野生型マウス（Ｗｉｌｄ－ｔｙｐｅ）のマクロファージはＣｐＧ　ＯＤＮに応答してＴＮＦα、ＩＬ－６及びＩＬ－１２を産生し、さらにＩＦＮγ及びＣｐＧ　ＯＤＮで刺激すると、ＴＮＦα、ＩＬ－６及びＩＬ－１２の産生量が増加することがわかった。しかし、ＴＬＲ９ノックアウトマウス（$TLR9^{-/-}$）由来のマクロファージは、ＩＦＮγの存在下でさえ、ＣｐＧ　ＯＤＮに対する応答において検出可能なレベルの炎症性サイトカインを産生していなかった。また、野生型マウス及びＴＬＲ９ノックアウトマウス由来のマクロファージは、ＬＰＳ又はＰＧＮに対する応答によりＴＮＦα、ＩＬ－６及びＩＬ－１２をほぼ同程度産生することがわかった（図５）。なお、それぞれの実験結果はｎ＝３の平均値を示す。図中のＮ．Ｄ．は検出できなかったことを示す。

【００５４】また、ＣｐＧ　ＯＤＮ又はＬＰＳに対する

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野生型マウス（Ｗｉｌｄ－ｔｙｐｅ）及びＴＬＲ９ノックアウトマウス（ＴＬＲ９$^{-/-}$）の脾臓細胞の応答性について調べてみた。それぞれのマウスの脾臓細胞（１×１０^{5}）を単離し、図６に示す各種濃度のＣｐＧ　ＯＤＮ又はＬＰＳにより９６ウェルプレート内で培養して脾臓細胞を刺激した。培養から４０時間後に１μＣｉの［^{3}Ｈ］－チミジン（デュポント社製）を添加して更に８時間培養し、［^{3}Ｈ］の摂取量をβシンチレーションカウンター（パッカード社製）で測定した（図６）。この結果から、野生型マウスの脾臓細胞では、ＣｐＧ　ＯＤＮやＬＰＳの投与量に依存して細胞増殖反応を促進していたが、ＴＬＲ９ノックアウトマウスの脾臓細胞では、いかなる濃度のＣｐＧ　ＯＤＮ刺激においてもＣｐＧ　ＯＤＮによる細胞増殖反応は見られなかった。また、ＣｐＧ　ＯＤＮに応答して、野生型マウス由来のＢ細胞表面の主要組織適合遺伝子複合体（ＭＨＣ）クラスIIの発現が増加した。しかし、ＴＬＲ９ノックアウトマウス由来のＢ細胞ではＣｐＧ　ＯＤＮに誘導されたＭＨＣクラスIIの発現の増加は見られなかった。以上のことから、ＴＬＲ９ノックアウトマウスのマクロファージやＢ細胞は、ＣｐＧ　ＯＤＮに対する応答性を特異的に欠如していることがわかった。

【００５５】次に、ＣｐＧ　ＯＤＮを含有するバクテリア由来ＤＮＡは樹状細胞を潜在的に刺激し、Ｔｈ１細胞の発達をサポートすることが知られている（EMBO J. 18, 6973-6982, 1999、J. Immunol. 161, 3042-3049, 1998、Proc. Natl. Acad. Sci. USA 96, 9305-9310, 1999）。そこでＣｐＧ　ＯＤＮ誘導サイトカインの産生と、骨髄由来の樹状細胞の表面分子のアップレギュレーションを分析した。野生型マウス（Ｗｉｌｄ－ｔｙｐｅ）又はＴＬＲ９ノックアウトマウス（ＴＬＲ９$^{-/-}$）の骨髄細胞を、１０ｎｇ／ｍｌのマウス顆粒球マクロファージ－コロニー刺激因子（Peprotech社製）を含む１０％のウシ胎仔血清を添加したＲＰＭＩ１６４０培地で培養し（J. Exp. Med. 176, 1693-1702, 1992）、培養後６日目に未成熟の樹状細胞を回収し、０．１μＭのＣｐＧ　ＯＤＮ又は０．１μｇ／ｍｌのＬＰＳの存在下若しくは非存在下において、１０％のウシ胎仔血清を添加したＲＰＭＩ１６４０培地中で２日間培養した。培養後、上清中のＩＬ－１２　ｐ４０の濃度をＥＬＩＳＡ法で測定した（図７）。この結果から、野生型マウス由来の樹状細胞はＣｐＧ　ＯＤＮに応答してＩＬ－１２を産生したが、ＴＬＲ９ノックアウトマウス由来の樹状細胞においては、ＣｐＧ　ＯＤＮはＩＬ－１２の産生を誘導しなかった。

【００５６】上記１０ｎｇ／ｍｌのマウス顆粒球マクロファージ－コロニー刺激因子（Peprotech社製）を含む１０％のウシ胎仔血清を添加したＲＰＭＩ１６４０培地で培養し、６日目に回収された樹状細胞を、ＣＤ４０、ＣＤ８０、ＣＤ８６及びＭＨＣクラスIIに対する、それぞれのビオチン化抗体により染色し、フィコエリトリン（ｐｈｙｃｏｅｒｙｔｈｒｉｎ：ＰＥ；ファーミンジェン社製）で標識したストレプトアビジンで発展させ、これらの細胞をセルクエストソフトウェア（ベクトンディッキンソン社製）により蛍光活性化セルソーターキャリバー（FACS Calibur）で分析した（図８）。この結果から、ＣｐＧ　ＯＤＮで刺激すると、野生型マウス由来の樹状細胞表面においては、ＣＤ４０、ＣＤ８０、ＣＤ８６及びＭＨＣクラスIIの発現を促進していたが、ＴＬＲ９ノックアウトマウス由来の樹状細胞表面では、ＣｐＧ　ＯＤＮに対する応答によりこれらの分子の発現を促進しなかった（図８）。ＬＰＳによる刺激では、野生型マウス由来の樹状細胞もＴＬＲ９ノックアウトマウス由来の樹状細胞も同様の応答がみられた。以上の結果から、ＴＬＲ９はＣｐＧ　ＯＤＮの細胞応答に不可欠な受容体であることがわかった。

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【００５７】実施例５（ＴＬＲ９ノックアウトマウス由来のマクロファージのＣｐＧ　ＯＤＮに対する応答によるＮＦ－κＢ、ＪＮＫ及びＩＲＡＫの活性化）

ＴＬＲのシグナルは、アダプター分子であるＭｙＤ８８を介してセリン／トレオニンキナーゼであるＩＲＡＫを活性化し、次いでＭＡＰキナーゼ及びＮＦ－κＢを活性化することが知られている（Immunity 11, 115-122, 1999）。そこでＣｐＧ　ＯＤＮが、かかる細胞内シグナル伝達分子を活性化するかどうかを調べてみた。実施例３により調製した野生型マウス及びＴＬＲ９ノックアウトマウスの腹腔マクロファージ（１×１０^{6}cells）を、１．０μＭのＣｐＧ　ＯＤＮ又は１．０μｇ／ｍｌのサルモネラ・ミネソタＲｅ－５９５のＬＰＳで図９に示された時間刺激し、各マウスのマクロファージから核蛋白質を抽出し、ＮＦ－κＢのＤＮＡ結合部位を含む特異的プローブといっしょにインキュベートし、電気泳動を行い、オートラジオグラフィーにより視覚化した（図９）。

【００５８】この結果から、ＣｐＧ　ＯＤＮで刺激すると、野生型マウス由来のマクロファージではＮＦ－κＢのＤＮＡ結合活性が増加するのに対し、ＴＬＲ９ノックアウトマウス由来のマクロファージではＮＦ－κＢのＤＮＡ結合活性は増加しなかった。ＴＬＲ９ノックアウトマウス由来のマクロファージをＬＰＳで刺激したものは、野生型マウス由来のマクロファージをＬＰＳで刺激したものと同様のＮＦ－κＢの活性化が見られた。以上の結果から、ＣｐＧ　ＯＤＮの誘導によるＮＦ－κＢの活性がＴＬＲ９ノックアウトマウス由来のマクロファージにおいて特異的に欠損していることがわかる。なお、図中の矢印はＮＦ－κＢと特異的プローブとの複合物の位置を示し、矢頭は特異的プローブのみの位置を示している。

【００５９】上記と同様に図１０又は図１１で示された時間、ＣｐＧ　ＯＤＮ又はＬＰＳで刺激した野生型マウ

ス及びＴＬＲ９ノックアウトマウスのマクロファージを、溶解緩衝液（最終濃度で１．０％のトリトンＸ－１００、１３７mMのＮａＣｌ、２０mMのトリスーＨＣｌ、５mMのＥＤＴＡ、１０％のグリセロール、１mMのＰＭＳＦ、２０μｇ／ｍｌのアプロチニン、２０μｇ／ｍｌのロイペプチン、１mMのＮａ₃ＶＯ₄及び１０mMのβ－グリセロリン酸を含有する緩衝液；ｐＨ８．０）中にて溶解し、この細胞溶解物を抗ＪＮＫ抗体（サンタクルス社製）又は抗ＩＲＡＫ抗体（林原生化学研究所株式会社製）で免疫沈降して、文献（Immunity 11, 115-122, 1999）記載のように、インビトロキナーゼアッセイを行い、ＧＳＴ－ｃ－Ｊｕｎ溶解蛋白質（ＧＳＴ－ｃ－Ｊｕｎ）を基質としたJNK活性及びＩＲＡＫの活性を測定した（図１０，１１における上段；ＧＳＴ－ｃ－Ｊｕｎ，Ａｕｔｏ）。

【００６０】また、上記細胞溶解物を、ＳＤＳ－ポリアクリルアミドゲル電気泳動により分離させ、ニトロセルロース膜に移し、この膜を抗ＪＮＫ抗体（サンタクルス社製）又は抗ＩＲＡＫ抗体（Transduction Laboratories社製）でブロットして、エンハンスド・ケミルミネッセンス装置（デュポント社製）を使用して視覚化した（図１０，１１における下段；ＷＢ）。以上の結果から、ＣｐＧ　ＯＤＮは野生型マウス由来のマクロファージのＪＮＫ及びＩＲＡＫを活性化するが、ＴＬＲ９ノックアウトマウス由来のマクロファージでは全く活性化しないことがわかった（図１０，１１）。したがって、ＣｐＧ　ＯＤＮを介する情報伝達はＴＬＲ９に依存していることがわかった。

【００６１】

【発明の効果】メチル化されていないＣｐＧモチーフを含有するバクテリア由来ＤＮＡは免疫細胞を非常に活性化し、Ｔｈ１の応答を誘導するが、そのバクテリア由来ＤＮＡを認識する受容体は知られていなかった。本発明により、細菌ＤＮＡの非メチル化ＣｐＧ配列を含むオリゴヌクレオチドの受容体が明らかとなったことから、非メチル化ＣｐＧ配列を有する細菌ＤＮＡを特異的に認識するＴＬＲファミリーのメンバー受容体タンパク質ＴＬＲ９や、それをコードする遺伝子ＤＮＡ等は、細菌性疾病等の診断や、治療に用いることができ、またＴＬＲ９ノックアウト動物を用いると、バクテリア由来ＤＮＡの分子レベルにおける作用機作を明らかにすることが可能となる。

【００６２】

【配列表】

SEQUENCE LISTING

<110> JAPAN SCIENCE AND TECHNOLOGY CORPORATION

<120> Specific receptor that recognizes bacterial DNA

<130> A031P63

<140>

<141>

<160> 5

<170> PatentIn Ver. 2.1

<210> 1

<211> 3257

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (107)..(3205)

<400> 1

ccgctgctgc ccctgtggga agggacctcg agtgtgaagc atcctcccct gtagctgctg 60

tccagtctgc ccgccagacc ctctggagaa gcccctgccc cccagc atg ggt ttc 115

Met Gly Phe

1

tgc cgc agc gcc ctg cac ccg ctg tct ctc ctg gtg cag gcc atc atg 163

Cys Arg Ser Ala Leu His Pro Leu Ser Leu Leu Val Gln Ala Ile Met

5 10 15

ctg gcc atg acc ctg gcc ctg ggt acc ttg cct gcc ttc cta ccc tgt 211

Leu Ala Met Thr Leu Ala Leu Gly Thr Leu Pro Ala Phe Leu Pro Cys

20 25 30 35

gag ctc cag ccc cac ggc ctg gtg aac tgc aac tgg ctg ttc ctg aag 259

Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu Phe Leu Lys

27　　28

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                40                  45                  50
tct gtg ccc cac ttc tcc atg gca gca ccc cgt ggc aat gtc acc agc   307
Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn Val Thr Ser
            55                  60                  65
ctt tcc ttg tcc tcc aac cgc atc cac cac ctc cat gat tct gac ttt   355
Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp Ser Asp Phe
        70                  75                  80
gcc cac ctg ccc agc ctg cgg cat ctc aac ctc aag tgg aac tgc ccg   403
Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp Asn Cys Pro
    85                  90                  95
ccg gtt ggc ctc agc ccc atg cac ttc ccc tgc cac atg acc atc gag   451
Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met Thr Ile Glu
100                 105                 110                 115
ccc agc acc ttc ttg gct gtg ccc acc ctg gaa gag cta aac ctg agc   499
Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu Asn Leu Ser
                120                 125                 130
tac aac aac atc atg act gtg cct gcg ctg ccc aaa tcc ctc ata tcc   547
Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser Leu Ile Ser
            135                 140                 145
ctg tcc ctc agc cat acc aac atc ctg atg cta gac tct gcc agc ctc   595
Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser Ala Ser Leu
        150                 155                 160
gcc ggc ctg cat gcc ctg cgc ttc cta ttc atg gac ggc aac tgt tat   643
Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly Asn Cys Tyr
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tac aag aac ccc tgc agg cag gca ctg gag gtg gcc ccg ggt gcc ctc   691
Tyr Lys Asn Pro Cys Arg Gln Ala Leu Glu Val Ala Pro Gly Ala Leu
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ctt ggc ctg ggc aac ctc acc cac ctg tca ctc aag tac aac aac ctc   739
Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr Asn Asn Leu
                200                 205                 210
act gtg gtg ccc cgc aac ctg cct tcc agc ctg gag tat ctg ctg ttg   787
Thr Val Val Pro Arg Asn Leu Pro Ser Ser Leu Glu Tyr Leu Leu Leu
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tcc tac aac cgc atc gtc aaa ctg gcg cct gag gac ctg gcc aat ctg   835
Ser Tyr Asn Arg Ile Val Lys Leu Ala Pro Glu Asp Leu Ala Asn Leu
        230                 235                 240
acc gcc ctg cgt gtg ctc gat gtg ggc gga aat tgc cgc cgc tgc gac   883
Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg Cys Asp
    245                 250                 255
cac gct ccc aac ccc tgc atg gag tgc cct cgt cac ttc ccc cag cta   931
His Ala Pro Asn Pro Cys Met Glu Cys Pro Arg His Phe Pro Gln Leu
260                 265                 270                 275
cat ccc gat acc ttc agc cac ctg agc cgt ctt gaa ggc ctg gtg ttg   979
His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly Leu Val Leu
                280                 285                 290
aag gac agt tct ctc tcc tgg ctg aat gcc agt tgg ttc cgt ggg ctg   1027
Lys Asp Ser Ser Leu Ser Trp Leu Asn Ala Ser Trp Phe Arg Gly Leu
            295                 300                 305
gga aac ctc cga gtg ctg gac ctg agt gag aac ttc ctc tac aaa tgc   1075
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Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr Lys Cys
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atc act aaa acc aag gcc ttc cag ggc cta aca cag ctg cgc aag ctt   1123
Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Thr Gln Leu Arg Lys Leu
    325                 330                 335
aac ctg tcc ttc aat tac caa aag agg gtg tcc ttt gcc cac ctg tct   1171
Asn Leu Ser Phe Asn Tyr Gln Lys Arg Val Ser Phe Ala His Leu Ser
340                 345                 350                 355
ctg gcc cct tcc ttc ggg agc ctg gtc gcc ctg aag gag ctg gac atg   1219
Leu Ala Pro Ser Phe Gly Ser Leu Val Ala Leu Lys Glu Leu Asp Met
                360                 365                 370
cac ggc atc ttc ttc cgc tca ctc gat gag acc acg ctc cgg cca ctg   1267
His Gly Ile Phe Phe Arg Ser Leu Asp Glu Thr Thr Leu Arg Pro Leu
            375                 380                 385
gcc cgc ctg ccc atg ctc cag act ctg cgt ctg cag atg aac ttc atc   1315
Ala Arg Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met Asn Phe Ile
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aac cag gcc cag ctc ggc atc ttc agg gcc ttc cct ggc ctg cgc tac   1363
Asn Gln Ala Gln Leu Gly Ile Phe Arg Ala Phe Pro Gly Leu Arg Tyr
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gtg gac ctg tcg gac aac cgc atc agc gga gct tcg gag ctg aca gcc   1411
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acc atg ggg gag gca gat gga ggg gag aag gtc tgg ctg cag cct ggg   1459
Thr Met Gly Glu Ala Asp Gly Gly Glu Lys Val Trp Leu Gln Pro Gly
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gac ctt gct ccg gcc cca gtg gac act ccc agc tct gaa gac ttc agg   1507
Asp Leu Ala Pro Ala Pro Val Asp Thr Pro Ser Ser Glu Asp Phe Arg
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ccc aac tgc agc acc ctc aac ttc acc ttg gat ctg tca cgg aac aac   1555
Pro Asn Cys Ser Thr Leu Asn Phe Thr Leu Asp Leu Ser Arg Asn Asn
        470                 475                 480
ctg gtg acc gtg cag ccg gag atg ttt gcc cag ctc tcg cac ctg cag   1603
Leu Val Thr Val Gln Pro Glu Met Phe Ala Gln Leu Ser His Leu Gln
    485                 490                 495
tgc ctg cgc ctg agc cac aac tgc atc tcg cag gca gtc aat ggc tcc   1651
Cys Leu Arg Leu Ser His Asn Cys Ile Ser Gln Ala Val Asn Gly Ser
500                 505                 510                 515
cag ttc ctg ccg ctg acc ggt ctg cag gtg cta gac ctg tcc cac aat   1699
Gln Phe Leu Pro Leu Thr Gly Leu Gln Val Leu Asp Leu Ser His Asn
                520                 525                 530
aag ctg gac ctc tac cac gag cac tca ttc acg gag cta cca cga ctg   1747
Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu Pro Arg Leu
            535                 540                 545
gag gcc ctg gac ctc agc tac aac agc cag ccc ttt ggc atg cag ggc   1795
Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly Met Gln Gly
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gtg ggc cac aac ttc agc ttc gtg gct cac ctg cgc acc ctg cgc cac   1843
Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr Leu Arg His
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ctc agc ctg gcc cac aac aac atc cac agc caa gtg tcc cag cag ctc   1891
Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser Gln Gln Leu
580                 585                 590                 595
tgc agt acg tcg ctg cgg gcc ctg gac ttc agc ggc aat gca ctg ggc   1939
Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn Ala Leu Gly
                600                 605                 610
cat atg tgg gcc gag gga gac ctc tat ctg cac ttc ttc caa ggc ctg   1987
His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe Gln Gly Leu
            615                 620                 625
agc ggt ttg atc tgg ctg gac ttg tcc cag aac cgc ctg cac acc ctc   2035
Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu His Thr Leu
        630                 635                 640
ctg ccc caa acc ctg cgc aac ctc ccc aag agc cta cag gtg ctg cgt   2083
Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln Val Leu Arg
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ctc cgt gac aat tac ctg gcc ttc ttt aag tgg tgg agc ctc cac ttc   2131
Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser Leu His Phe
660                 665                 670                 675
ctg ccc aaa ctg gaa gtc ctc gac ctg gca gga aac cag ctg aag gcc   2179
Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Gln Leu Lys Ala
                680                 685                 690
ctg acc aat ggc agc ctg cct gct ggc acc cgg ctc cgg agg ctg gat   2227
Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg Arg Leu Asp
            695                 700                 705
gtc agc tgc aac agc atc agc ttc gtg gcc ccc ggc ttc ttt tcc aag   2275
Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe Phe Ser Lys
        710                 715                 720
gcc aag gag ctg cga gag ctc aac ctt agc gcc aac gcc ctc aag aca   2323
Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala Leu Lys Thr
    725                 730                 735
gtg gac cac tcc tgg ttt ggg ccc ctg gcg agt gcc ctg caa ata cta   2371
Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu Gln Ile Leu
740                 745                 750                 755
gat gta agc gcc aac cct ctg cac tgc gcc tgt ggg gcg gcc ttt atg   2419
Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe Met
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gac ttc ctg ctg gag gtg cag gct gcc gtg ccc ggt ctg ccc agc cgg   2467
Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu Pro Ser Arg
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gtg aag tgt ggc agt ccg ggc cag ctc cag ggc ctc agc atc ttt gca   2515
Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser Ile Phe Ala
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cag gac ctg cgc ctc tgc ctg gat gag gcc ctc tcc tgg gac tgt ttc   2563
Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp Asp Cys Phe
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gcc ctc tcg ctg ctg gct gtg gct ctg ggc ctg ggt gtg ccc atg ctg   2611
Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val Pro Met Leu
820                 825                 830                 835
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His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His Leu Cys Leu
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33 34

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gcc tgg ctt ccc tgg cgg ggg cgg caa agt ggg cga gat gag gat gcc   2707
Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp Glu Asp Ala
          855                 860                 865
ctg ccc tac gat gcc ttc gtg gtc ttc gac aaa acg cag agc gca gtg   2755
Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln Ser Ala Val
       870                 875                 880
gca gac tgg gtg tac aac gag ctt cgg ggg cag ctg gag gag tgc cgt   2803
Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu Glu Cys Arg
    885                 890                 895
ggg cgc tgg gca ctc cgc ctg tgc ctg gag gaa cgc gac tgg ctg cct   2851
Gly Arg Trp Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp Trp Leu Pro
900                 905                 910                 915
ggc aaa acc ctc ttt gag aac ctg tgg gcc tcg gtc tat ggc agc cgc   2899
Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr Gly Ser Arg
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aag acg ctg ttt gtg ctg gcc cac acg gac cgg gtc agt ggt ctc ttg   2947
Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser Gly Leu Leu
          935                 940                 945
cgc gcc agc ttc ctg ctg gcc cag cag cgc ctg ctg gag gac cgc aag   2995
Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys
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gac gtc gtg gtg ctg gtg atc ctg agc cct gac ggc cgc cgc tcc cgc   3043
Asp Val Val Val Leu Val Ile Leu Ser Pro Asp Gly Arg Arg Ser Arg
    965                 970                 975
tac gtg cgg ctg cgc cag cgc ctc tgc cgc cag agt gtc ctc ctc tgg   3091
Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val Leu Leu Trp
980                 985                 990                 995
ccc cac cag ccc agt ggt cag cgc agc ttc tgg gcc cag ctg ggc atg   3139
Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln Leu Gly Met
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gcc ctg acc agg gac aac cac cac ttc tat aac cgg aac ttc tgc cag   3187
Ala Leu Thr Arg Asp Asn His His Phe Tyr Asn Arg Asn Phe Cys Gln
          1015                1020                1025
gga ccc acg gcc gaa tag ccgtgagccg gaatcctgca cggtgccacc          3235
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Leu Pro Cys Glu Leu Gln Pro His Gly Leu Val Asn Cys Asn Trp Leu
        35                  40                  45
Phe Leu Lys Ser Val Pro His Phe Ser Met Ala Ala Pro Arg Gly Asn
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35　　36

50　　55　　60

Val Thr Ser Leu Ser Leu Ser Ser Asn Arg Ile His His Leu His Asp
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Ser Asp Phe Ala His Leu Pro Ser Leu Arg His Leu Asn Leu Lys Trp
85　　90　　95
Asn Cys Pro Pro Val Gly Leu Ser Pro Met His Phe Pro Cys His Met
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Thr Ile Glu Pro Ser Thr Phe Leu Ala Val Pro Thr Leu Glu Glu Leu
115　　120　　125
Asn Leu Ser Tyr Asn Asn Ile Met Thr Val Pro Ala Leu Pro Lys Ser
130　　135　　140
Leu Ile Ser Leu Ser Leu Ser His Thr Asn Ile Leu Met Leu Asp Ser
145　　150　　155　　160
Ala Ser Leu Ala Gly Leu His Ala Leu Arg Phe Leu Phe Met Asp Gly
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Asn Cys Tyr Tyr Lys Asn Pro Cys Arg Gln Ala Leu Glu Val Ala Pro
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Gly Ala Leu Leu Gly Leu Gly Asn Leu Thr His Leu Ser Leu Lys Tyr
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Asn Asn Leu Thr Val Val Pro Arg Asn Leu Pro Ser Ser Leu Glu Tyr
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Leu Leu Leu Ser Tyr Asn Arg Ile Val Lys Leu Ala Pro Glu Asp Leu
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Ala Asn Leu Thr Ala Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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Arg Cys Asp His Ala Pro Asn Pro Cys Met Glu Cys Pro Arg His Phe
260　　265　　270
Pro Gln Leu His Pro Asp Thr Phe Ser His Leu Ser Arg Leu Glu Gly
275　　280　　285
Leu Val Leu Lys Asp Ser Ser Leu Ser Trp Leu Asn Ala Ser Trp Phe
290　　295　　300
Arg Gly Leu Gly Asn Leu Arg Val Leu Asp Leu Ser Glu Asn Phe Leu
305　　310　　315　　320
Tyr Lys Cys Ile Thr Lys Thr Lys Ala Phe Gln Gly Leu Thr Gln Leu
325　　330　　335
Arg Lys Leu Asn Leu Ser Phe Asn Tyr Gln Lys Arg Val Ser Phe Ala
340　　345　　350
His Leu Ser Leu Ala Pro Ser Phe Gly Ser Leu Val Ala Leu Lys Glu
355　　360　　365
Leu Asp Met His Gly Ile Phe Phe Arg Ser Leu Asp Glu Thr Thr Leu
370　　375　　380
Arg Pro Leu Ala Arg Leu Pro Met Leu Gln Thr Leu Arg Leu Gln Met
385　　390　　395　　400
Asn Phe Ile Asn Gln Ala Gln Leu Gly Ile Phe Arg Ala Phe Pro Gly
405　　410　　415
Leu Arg Tyr Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Ala Ser Glu
420　　425　　430
Leu Thr Ala Thr Met Gly Glu Ala Asp Gly Gly Glu Lys Val Trp Leu
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Gln Pro Gly Asp Leu Ala Pro Ala Pro Val Asp Thr Pro Ser Ser Glu

450　　455　　460
Asp Phe Arg Pro Asn Cys Ser Thr Leu Asn Phe Thr Leu Asp Leu Ser
465　　470　　475　　480
Arg Asn Asn Leu Val Thr Val Gln Pro Glu Met Phe Ala Gln Leu Ser
485　　490　　495
His Leu Gln Cys Leu Arg Leu Ser His Asn Cys Ile Ser Gln Ala Val
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Asn Gly Ser Gln Phe Leu Pro Leu Thr Gly Leu Gln Val Leu Asp Leu
515　　520　　525
Ser His Asn Lys Leu Asp Leu Tyr His Glu His Ser Phe Thr Glu Leu
530　　535　　540
Pro Arg Leu Glu Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Gly
545　　550　　555　　560
Met Gln Gly Val Gly His Asn Phe Ser Phe Val Ala His Leu Arg Thr
565　　570　　575
Leu Arg His Leu Ser Leu Ala His Asn Asn Ile His Ser Gln Val Ser
580　　585　　590
Gln Gln Leu Cys Ser Thr Ser Leu Arg Ala Leu Asp Phe Ser Gly Asn
595　　600　　605
Ala Leu Gly His Met Trp Ala Glu Gly Asp Leu Tyr Leu His Phe Phe
610　　615　　620
Gln Gly Leu Ser Gly Leu Ile Trp Leu Asp Leu Ser Gln Asn Arg Leu
625　　630　　635　　640
His Thr Leu Leu Pro Gln Thr Leu Arg Asn Leu Pro Lys Ser Leu Gln
645　　650　　655
Val Leu Arg Leu Arg Asp Asn Tyr Leu Ala Phe Phe Lys Trp Trp Ser
660　　665　　670
Leu His Phe Leu Pro Lys Leu Glu Val Leu Asp Leu Ala Gly Asn Gln
675　　680　　685
Leu Lys Ala Leu Thr Asn Gly Ser Leu Pro Ala Gly Thr Arg Leu Arg
690　　695　　700
Arg Leu Asp Val Ser Cys Asn Ser Ile Ser Phe Val Ala Pro Gly Phe
705　　710　　715　　720
Phe Ser Lys Ala Lys Glu Leu Arg Glu Leu Asn Leu Ser Ala Asn Ala
725　　730　　735
Leu Lys Thr Val Asp His Ser Trp Phe Gly Pro Leu Ala Ser Ala Leu
740　　745　　750
Gln Ile Leu Asp Val Ser Ala Asn Pro Leu His Cys Ala Cys Gly Ala
755　　760　　765
Ala Phe Met Asp Phe Leu Leu Glu Val Gln Ala Ala Val Pro Gly Leu
770　　775　　780
Pro Ser Arg Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Leu Ser
785　　790　　795　　800
Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Ala Leu Ser Trp
805　　810　　815
Asp Cys Phe Ala Leu Ser Leu Leu Ala Val Ala Leu Gly Leu Gly Val
820　　825　　830
Pro Met Leu His His Leu Cys Gly Trp Asp Leu Trp Tyr Cys Phe His
835　　840　　845
Leu Cys Leu Ala Trp Leu Pro Trp Arg Gly Arg Gln Ser Gly Arg Asp

39 40

850 855 860

Glu Asp Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Thr Gln
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Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Gly Gln Leu Glu
885 890 895
Glu Cys Arg Gly Arg Trp Ala Leu Arg Leu Cys Leu Glu Glu Arg Asp
900 905 910
Trp Leu Pro Gly Lys Thr Leu Phe Glu Asn Leu Trp Ala Ser Val Tyr
915 920 925
Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
930 935 940
Gly Leu Leu Arg Ala Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945 950 955 960
Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Ser Pro Asp Gly Arg
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Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
980 985 990
Leu Leu Trp Pro His Gln Pro Ser Gly Gln Arg Ser Phe Trp Ala Gln
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Met Val Leu
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cgt cga agg act ctg cac ccc ttg tcc ctc ctg gta cag gct gca gtg 163
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Leu Ala Glu Thr Leu Ala Leu Gly Thr Leu Pro Ala Phe Leu Pro Cys
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Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu Phe Leu Lys
40 45 50
tct gta ccc cgt ttc tct gcg gca gca tcc tgc tcc aac atc acc cgc 307
Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn Ile Thr Arg
55 60 65
ctc tcc ttg atc tcc aac cgt atc cac cac ctg cac aac tcc gac ttc 355
Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn Ser Asp Phe
70 75 80

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Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp Asn Cys Pro
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ccc act ggc ctt agc ccc ttg cac ttc tct tgc cac atg acc att gag   451
Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met Thr Ile Glu
100                 105                 110                 115
ccc aga acc ttc ctg gct atg cgt aca ctg gag gag ctg aac ctg agc   499
Pro Arg Thr Phe Leu Ala Met Arg Thr Leu Glu Glu Leu Asn Leu Ser
                120                 125                 130
tat aat ggt atc acc act gtg ccc cga ctg ccc agc tcc ctg gtg aat   547
Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser Leu Val Asn
            135                 140                 145
ctg agc ctg agc cac acc aac atc ctg gtt cta gat gct aac agc ctc   595
Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala Asn Ser Leu
        150                 155                 160
gcc ggc cta tac agc ctg cgc gtt ctc ttc atg gac ggg aac tgc tac   643
Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly Asn Cys Tyr
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Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro Gly Ala Leu
180                 185                 190                 195
ctg ggc ctg agc aat ctc acc cat ctg tct gtg aag tat aac aac ctc   739
Leu Gly Leu Ser Asn Leu Thr His Leu Ser Val Lys Tyr Asn Asn Leu
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Thr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr Leu Leu Val
            215                 220                 225
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Ser Tyr Asn Leu Ile Val Lys Leu Gly Pro Glu Asp Leu Ala Asn Leu
        230                 235                 240
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Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg Arg Cys Asp
    245                 250                 255
cat gcc ccc aat ccc tgt ata gaa tgt ggc caa aag tcc ctc cac ctg   931
His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser Leu His Leu
260                 265                 270                 275
cac cct gag acc ttc cat cac ctg agc cat ctg gaa ggc ctg gtg ctg   979
His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly Leu Val Leu
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Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu Tyr Glu Ser
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Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu Arg Lys Leu
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aac ctg tcc ttc aat tac cgc aag aag gta tcc ttt gcc cgc ctc cac   1171
Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala Arg Leu His
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43　　44

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360　　365　　370

aac ggc atc ttc ttc cgc tcg ctc aac aag tac acg ctc aga tgg ctg　1267
Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu Arg Trp Leu
375　　380　　385

gcc gat ctg ccc aaa ctc cac act ctg cat ctt caa atg aac ttc atc　1315
Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met Asn Phe Ile
390　　395　　400

aac cag gca cag ctc agc atc ttt ggt acc ttc cga gcc ctt cgc ttt　1363
Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala Leu Arg Phe
405　　410　　415

gtg gac ttg tca gac aat cgc atc agt ggg cct tca acg ctg tca gaa　1411
Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr Leu Ser Glu
420　　425　　430　　435

gcc acc cct gaa gag gca gat gat gca gag cag gag gag ctg ttg tct　1459
Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu Leu Leu Ser
440　　445　　450

gcg gat cct cac cca gct cca ctg agc acc cct gct tct aag aac ttc　1507
Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser Lys Asn Phe
455　　460　　465

atg gac agg tgt aag aac ttc aag ttc acc atg gac ctg tct cgg aac　1555
Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu Ser Arg Asn
470　　475　　480

aac ctg gtg act atc aag cca gag atg ttt gtc aat ctc tca cgc ctc　1603
Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu Ser Arg Leu
485　　490　　495

cag tgt ctt agc ctg agc cac aac tcc att gca cag gct gtc aat ggc　1651
Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala Val Asn Gly
500　　505　　510　　515

tct cag ttc ctg ccg ctg act aat ctg cag gtg ctg gac ctg tcc cat　1699
Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp Leu Ser His
520　　525　　530

aac aaa ctg gac ttg tac cac tgg aaa tcg ttc agt gag cta cca cag　1747
Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu Leu Pro Gln
535　　540　　545

ttg cag gcc ctg gac ctg agc tac aac agc cag ccc ttt agc atg aag　1795
Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe Ser Met Lys
550　　555　　560

ggt ata ggc cac aat ttc agt ttt gtg gcc cat ctg tcc atg cta cac　1843
Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser Met Leu His
565　　570　　575

agc ctt agc ctg gca cac aat gac att cat acc cgt gtg tcc tca cat　1891
Ser Leu Ser Leu Ala His Asn Asp Ile His Thr Arg Val Ser Ser His
580　　585　　590　　595

ctc aac agc aac tca gtg agg ttt ctt gac ttc agc ggc aac ggt atg　1939
Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly Asn Gly Met
600　　605　　610

ggc cgc atg tgg gat gag ggg ggc ctt tat ctc cat ttc ttc caa ggc　1987

45　　46

Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe Phe Gln Gly
615　620　625

ctg agt ggc ctg ctg aag ctg gac ctg tct caa aat aac ctg cat atc　2035
Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn Leu His Ile
630　635　640

ctc cgg ccc cag aac ctt gac aac ctc ccc aag agc ctg aag ctg ctg　2083
Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu Lys Leu Leu
645　650　655

agc ctc cga gac aac tac cta tct ttc ttt aac tgg acc agt ctg tcc　2131
Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr Ser Leu Ser
660　665　670　675

ttc ctg ccc aac ctg gaa gtc cta gac ctg gca ggc aac cag cta aag　2179
Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn Gln Leu Lys
680　685　690

gcc ctg acc aat ggc acc ctg cct aat ggc acc ctc ctc cag aaa ctg　2227
Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu Gln Lys Leu
695　700　705

gat gtc agc agc aac agt atc gtc tct gtg gtc cca gcc ttc ttc gct　2275
Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala Phe Phe Ala
710　715　720

ctg gcg gtc gag ctg aaa gag gtc aac ctc agc cac aac att ctc aag　2323
Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn Ile Leu Lys
725　730　735

acg gtg gat cgc tcc tgg ttt ggg ccc att gtg atg aac ctg aca gtt　2371
Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn Leu Thr Val
740　745　750　755

cta gac gtg aga agc aac cct ctg cac tgt gcc tgt ggg gca gcc ttc　2419
Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly Ala Ala Phe
760　765　770

gta gac tta ctg ttg gag gtg cag acc aag gtg cct ggc ctg gct aat　2467
Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly Leu Ala Asn
775　780　785

ggt gtg aag tgt ggc agc ccc ggc cag ctg cag ggc cgt agc atc ttc　2515
Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg Ser Ile Phe
790　795　800

gca cag gac ctg cgg ctg tgc ctg gat gag gtc ctc tct tgg gac tgc　2563
Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser Trp Asp Cys
805　810　815

ttt ggc ctt tca ctc ttg gct gtg gcc gtg ggc atg gtg gtg cct ata　2611
Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val Val Pro Ile
820　825　830　835

ctg cac cat ctc tgc ggc tgg gac gtc tgg tac tgt ttt cat ctg tgc　2659
Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe His Leu Cys
840　845　850

ctg gca tgg cta cct ttg ctg gcc cgc agc cga cgc agc gcc caa gct　2707
Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser Ala Gln Ala
855　860　865

ctc ccc tat gat gcc ttc gtg gtg ttc gat aag gca cag agc gca gtt　2755
Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln Ser Ala Val
870　875　880

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ggt cgc cga gcc cta cgc ttg tgt ctg gag gac cga gat tgg ctg cct   2851
Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp Trp Leu Pro
900                 905                 910                 915
ggc cag acg ctc ttc gag aac ctc tgg gct tcc atc tat ggg agc cgc   2899
Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr Gly Ser Arg
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aag act cta ttt gtg ctg gcc cac acg gac cgc gtc agt ggc ctc ctg   2947
Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser Gly Leu Leu
            935                 940                 945
cgc acc agc ttc ctg ctg gct cag cag cgc ctg ttg gaa gac cgc aag   2995
Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu Asp Arg Lys
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gac gtg gtg gtg ttg gtg atc ctg cgt ccg gat gcc cac cgc tcc cgc   3043
Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His Arg Ser Arg
    965                 970                 975
tat gtg cga ctg cgc cag cgt ctc tgc cgc cag agt gtg ctc ttc tgg   3091
Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val Leu Phe Trp
980                 985                 990                 995
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Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln Leu Ser Thr
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gcc ctg act agg gac aac cgc cac ttc tat aac cag aac ttc tgc cgg   3187
Ala Leu Thr Arg Asp Asn Arg His Phe Tyr Asn Gln Asn Phe Cys Arg
            1015                1020                1025
gga cct aca gca gaa tag ctcagagcaa cagctggaaa cagctgcatc          3235
Gly Pro Thr Ala Glu
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            20                  25                  30
Leu Pro Cys Glu Leu Lys Pro His Gly Leu Val Asp Cys Asn Trp Leu
        35                  40                  45
Phe Leu Lys Ser Val Pro Arg Phe Ser Ala Ala Ala Ser Cys Ser Asn
    50                  55                  60
Ile Thr Arg Leu Ser Leu Ile Ser Asn Arg Ile His His Leu His Asn
65                  70                  75                  80
Ser Asp Phe Val His Leu Ser Asn Leu Arg Gln Leu Asn Leu Lys Trp
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49　　50

85　　90　　95

Asn Cys Pro Pro Thr Gly Leu Ser Pro Leu His Phe Ser Cys His Met
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Thr Ile Glu Pro Arg Thr Phe Leu Ala Met Arg Thr Leu Glu Glu Leu
115　　120　　125
Asn Leu Ser Tyr Asn Gly Ile Thr Thr Val Pro Arg Leu Pro Ser Ser
130　　135　　140
Leu Val Asn Leu Ser Leu Ser His Thr Asn Ile Leu Val Leu Asp Ala
145　　150　　155　　160
Asn Ser Leu Ala Gly Leu Tyr Ser Leu Arg Val Leu Phe Met Asp Gly
165　　170　　175
Asn Cys Tyr Tyr Lys Asn Pro Cys Thr Gly Ala Val Lys Val Thr Pro
180　　185　　190
Gly Ala Leu Leu Gly Leu Ser Asn Leu Thr His Leu Ser Val Lys Tyr
195　　200　　205
Asn Asn Leu Thr Lys Val Pro Arg Gln Leu Pro Pro Ser Leu Glu Tyr
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Ala Asn Leu Thr Ser Leu Arg Val Leu Asp Val Gly Gly Asn Cys Arg
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Arg Cys Asp His Ala Pro Asn Pro Cys Ile Glu Cys Gly Gln Lys Ser
260　　265　　270
Leu His Leu His Pro Glu Thr Phe His His Leu Ser His Leu Glu Gly
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Gln Gly Leu Val Asn Leu Ser Val Leu Asp Leu Ser Glu Asn Phe Leu
305　　310　　315　　320
Tyr Glu Ser Ile Asn His Thr Asn Ala Phe Gln Asn Leu Thr Arg Leu
325　　330　　335
Arg Lys Leu Asn Leu Ser Phe Asn Tyr Arg Lys Lys Val Ser Phe Ala
340　　345　　350
Arg Leu His Leu Ala Ser Ser Phe Lys Asn Leu Val Ser Leu Gln Glu
355　　360　　365
Leu Asn Met Asn Gly Ile Phe Phe Arg Ser Leu Asn Lys Tyr Thr Leu
370　　375　　380
Arg Trp Leu Ala Asp Leu Pro Lys Leu His Thr Leu His Leu Gln Met
385　　390　　395　　400
Asn Phe Ile Asn Gln Ala Gln Leu Ser Ile Phe Gly Thr Phe Arg Ala
405　　410　　415
Leu Arg Phe Val Asp Leu Ser Asp Asn Arg Ile Ser Gly Pro Ser Thr
420　　425　　430
Leu Ser Glu Ala Thr Pro Glu Glu Ala Asp Asp Ala Glu Gln Glu Glu
435　　440　　445
Leu Leu Ser Ala Asp Pro His Pro Ala Pro Leu Ser Thr Pro Ala Ser
450　　455　　460
Lys Asn Phe Met Asp Arg Cys Lys Asn Phe Lys Phe Thr Met Asp Leu
465　　470　　475　　480
Ser Arg Asn Asn Leu Val Thr Ile Lys Pro Glu Met Phe Val Asn Leu

485　　490　　495
Ser Arg Leu Gln Cys Leu Ser Leu Ser His Asn Ser Ile Ala Gln Ala
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Val Asn Gly Ser Gln Phe Leu Pro Leu Thr Asn Leu Gln Val Leu Asp
515　　520　　525
Leu Ser His Asn Lys Leu Asp Leu Tyr His Trp Lys Ser Phe Ser Glu
530　　535　　540
Leu Pro Gln Leu Gln Ala Leu Asp Leu Ser Tyr Asn Ser Gln Pro Phe
545　　550　　555　　560
Ser Met Lys Gly Ile Gly His Asn Phe Ser Phe Val Ala His Leu Ser
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Ser Ser His Leu Asn Ser Asn Ser Val Arg Phe Leu Asp Phe Ser Gly
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Asn Gly Met Gly Arg Met Trp Asp Glu Gly Gly Leu Tyr Leu His Phe
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Phe Gln Gly Leu Ser Gly Leu Leu Lys Leu Asp Leu Ser Gln Asn Asn
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Leu His Ile Leu Arg Pro Gln Asn Leu Asp Asn Leu Pro Lys Ser Leu
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Lys Leu Leu Ser Leu Arg Asp Asn Tyr Leu Ser Phe Phe Asn Trp Thr
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Ser Leu Ser Phe Leu Pro Asn Leu Glu Val Leu Asp Leu Ala Gly Asn
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Gln Leu Lys Ala Leu Thr Asn Gly Thr Leu Pro Asn Gly Thr Leu Leu
690　　695　　700
Gln Lys Leu Asp Val Ser Ser Asn Ser Ile Val Ser Val Val Pro Ala
705　　710　　715　　720
Phe Phe Ala Leu Ala Val Glu Leu Lys Glu Val Asn Leu Ser His Asn
725　　730　　735
Ile Leu Lys Thr Val Asp Arg Ser Trp Phe Gly Pro Ile Val Met Asn
740　　745　　750
Leu Thr Val Leu Asp Val Arg Ser Asn Pro Leu His Cys Ala Cys Gly
755　　760　　765
Ala Ala Phe Val Asp Leu Leu Leu Glu Val Gln Thr Lys Val Pro Gly
770　　775　　780
Leu Ala Asn Gly Val Lys Cys Gly Ser Pro Gly Gln Leu Gln Gly Arg
785　　790　　795　　800
Ser Ile Phe Ala Gln Asp Leu Arg Leu Cys Leu Asp Glu Val Leu Ser
805　　810　　815
Trp Asp Cys Phe Gly Leu Ser Leu Leu Ala Val Ala Val Gly Met Val
820　　825　　830
Val Pro Ile Leu His His Leu Cys Gly Trp Asp Val Trp Tyr Cys Phe
835　　840　　845
His Leu Cys Leu Ala Trp Leu Pro Leu Leu Ala Arg Ser Arg Arg Ser
850　　855　　860
Ala Gln Ala Leu Pro Tyr Asp Ala Phe Val Val Phe Asp Lys Ala Gln
865　　870　　875　　880
Ser Ala Val Ala Asp Trp Val Tyr Asn Glu Leu Arg Val Arg Leu Glu

53　　54

885　　890　　895
Glu Arg Arg Gly Arg Arg Ala Leu Arg Leu Cys Leu Glu Asp Arg Asp
900　　905　　910
Trp Leu Pro Gly Gln Thr Leu Phe Glu Asn Leu Trp Ala Ser Ile Tyr
915　　920　　925
Gly Ser Arg Lys Thr Leu Phe Val Leu Ala His Thr Asp Arg Val Ser
930　　935　　940
Gly Leu Leu Arg Thr Ser Phe Leu Leu Ala Gln Gln Arg Leu Leu Glu
945　　950　　955　　960
Asp Arg Lys Asp Val Val Val Leu Val Ile Leu Arg Pro Asp Ala His
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Arg Ser Arg Tyr Val Arg Leu Arg Gln Arg Leu Cys Arg Gln Ser Val
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Leu Phe Trp Pro Gln Gln Pro Asn Gly Gln Gly Gly Phe Trp Ala Gln
995　　1000　　1005
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【図面の簡単な説明】

【図１】本発明のＴＬＲ９ノックアウトマウスと野生型マウスの遺伝子地図を示す図である。

【図２】本発明のＴＬＲ９ノックアウトマウスのサザンブロット分析の結果を示す図である。

【図３】本発明のＴＬＲ９ノックアウトマウスの脾臓細胞におけるノーザンブロット分析の結果を示す図である。

【図４】本発明のＴＬＲ９ノックアウトマウスと野生型マウスのアミノ酸配列の比較結果を示す図である。

【図５】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ、ＰＧＮ又はＬＰＳ誘導によるＴＮＦα、ＩＬ－６又はＩＬ１２の産生量の結果を示す図である。

【図６】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ又はＬＰＳ誘導による細胞増殖応答の結果を示す図である。

【図７】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ又はＬＰＳ誘導によるＩＬ－１２の産生量の結果を示す図である。

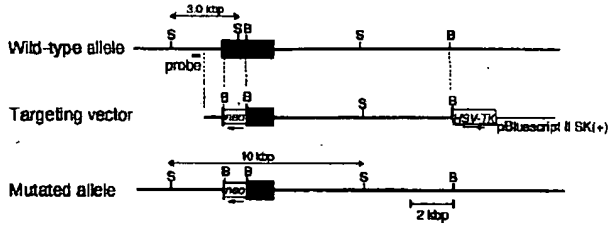
【図８】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ又はＬＰＳ誘導によるＣＤ４０、ＣＤ８０、ＣＤ８６及びＭＨＣクラスIIの発現量の結果を示す図である。

【図９】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ又はＬＰＳ誘導によるＮＦ－κＢの活性化の結果を示す図である。

【図１０】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ又はＬＰＳ誘導によるＪＮＫの活性化の結果を示す図である。

【図１１】本発明のＴＬＲ９ノックアウトマウス及び野生型マウスにおけるＣｐＧ　ＯＤＮ又はＬＰＳ誘導によるＩＲＡＫの活性化の結果を示す図である。

【図１】



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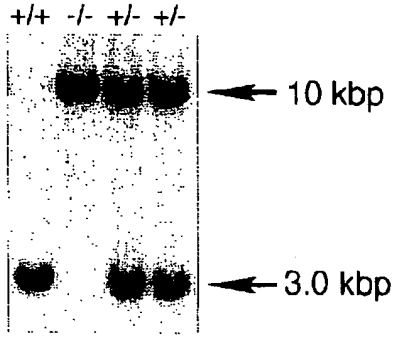

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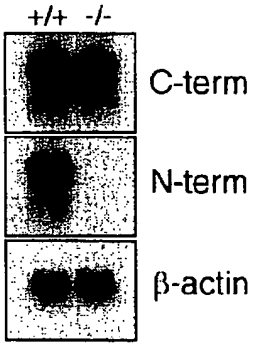

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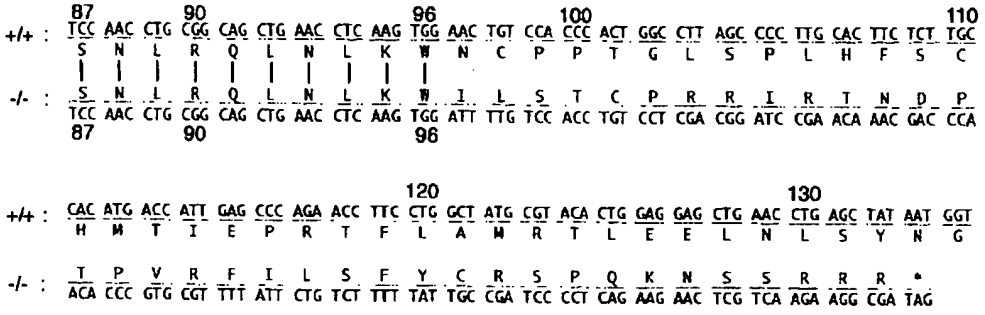

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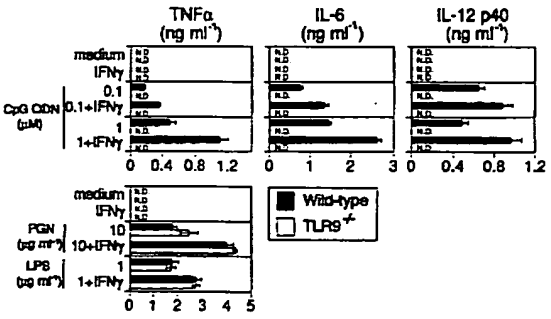

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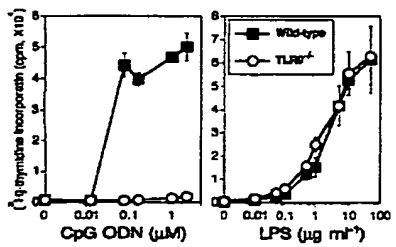

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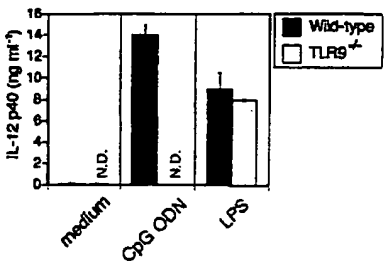
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N.D.
medium
CpG ODN
LPS

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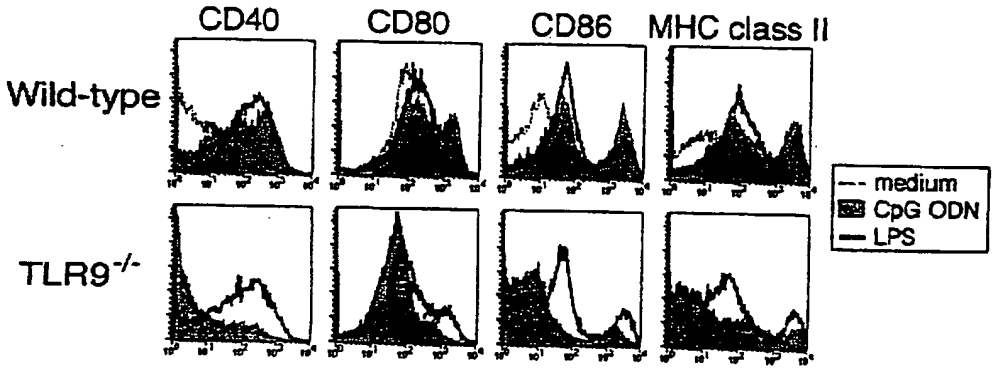
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MHC class II
Wild-type
TLR9-/-
medium
CpG ODN
LPS

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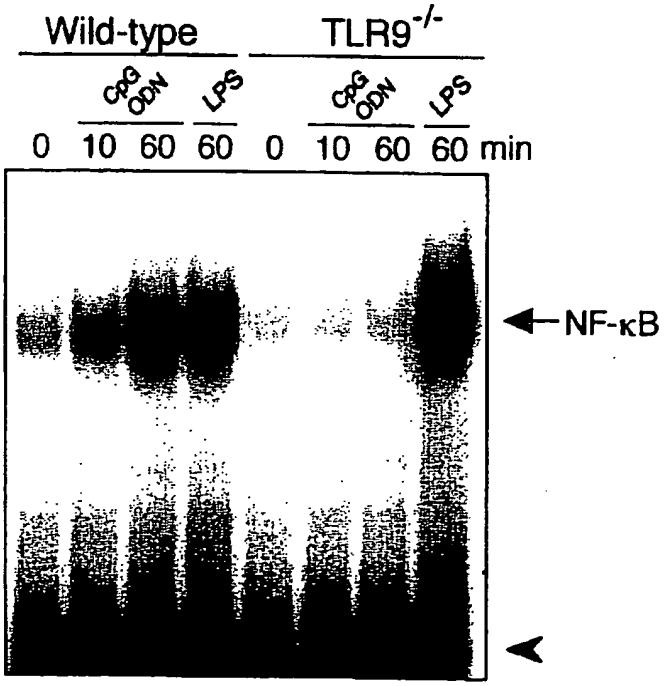
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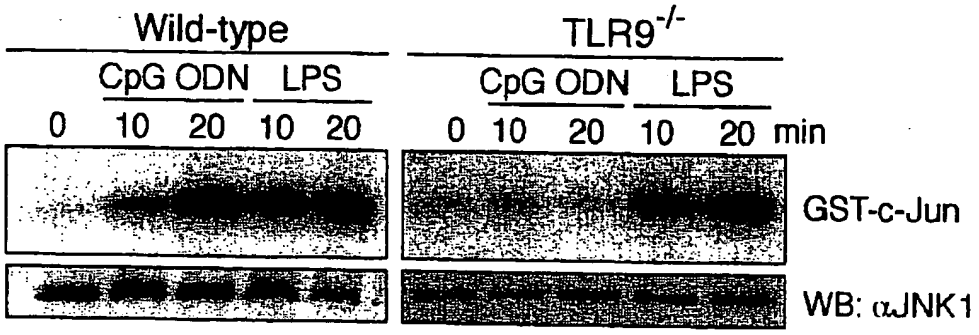

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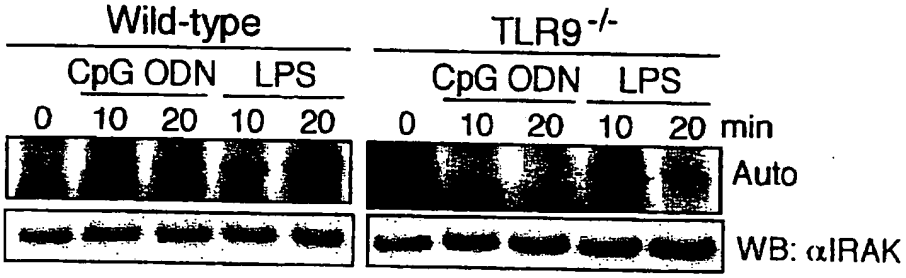

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A61P 37/08		C07K 16/28		4C084
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16/28		1/19		
C12N 1/15		1/21		
1/19		C12Q 1/68	A	
1/21		G01N 33/15	Z	
5/10		33/50	Z	
C12Q 1/68		33/566		
G01N 33/15		33/577	B	
33/50		C12P 21/08		
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AA91X AA92X AB01 AB05
AC10 BA02 CA24 CA25 CA43
4C084 AA01 AA17 BA44 ZB132
ZB262 ZB352
4H045 AA10 AA11 AA20 AA30 BA10
CA40 DA50 EA50 EA52 FA72
FA74